

Should we publicise those experiments?

THE vigorous debate, started several weeks ago by the appeal from a panel of the National Academy of Sciences for self-restraint in pursuing research on certain types of plasmid engineering, does not as yet show any sign of waning. True, the number of scientists technically qualified to appreciate the risks involved and weigh them against other considerations is relatively small, but each of them is going to wish to make a very personal contribution to the discussion since it probably concerns his way of going about research in the future. Further, there are many more scientists who may understand little or nothing of the technical content of the appeal but who can envisage quite well the sort of feelings that are aroused when a distinguished group of scientists proposes a halt to certain research work.

One of the ramifications of the proposed ban is that scientific journals might wish to take note of it in their publication policy. Thus David Baltimore, one of the NAS committee members, said "editorial boards . . . will probably think twice about publishing research papers derived from experiments covered by the embargo", and Michael Stoker, Director of the Imperial Cancer Research Fund, wrote "editors . . . will also need to consider their policies in the face of wide support for a moratorium".

We are obviously capable of speaking only for *Nature* but it is our opinion that any policy that tries to eliminate from the journal reports of experiments that are believed—even widely believed—to be potentially dangerous would be quite wrong. We are unsure that editors of more specialised journals would necessarily feel similarly.

There are two reasons why we feel that *Nature* should not take a stand against such papers. The first is that asking a referee to remark on the morality of a paper (assuming that it is not a question of legality) cannot but lead to impossible situations. Should reports of nuclear fission have been called 'potentially dangerous' and in bad taste? Should scientific work emerging from nuclear detonations or weather modification be kept out of the literature? Is the administering of electric shocks to animals cruel? These are not questions which can safely be left to a referee with his own personal predilections, or even an editor with his, and to attempt to do so by putting an X in the box if you believe the experiment should never have been done in the first place is rather like asking a judge to deliver his verdict only after he has factored in to his decisions his own feelings about whether the law was a good one to start with. The community can either legislate against certain experiments or it can attempt to persuade practitioners to desist; if it tries to put the onus on an individual to decide for the

community, it can hardly be surprised if the result is often quirky.

The second reason why we would not automatically decline papers describing things of which many would disapprove is that it is surely more important that these things be given publicity than that they be pushed into the underground.

This hardly means that we would actively solicit such manuscripts nor that we would be more likely to accept them in order to take advantage of their obviously unusual character. But if a scientist has chosen to ignore a widespread call then a case could occasionally be made for drawing the community's attention to who he is and what he is doing. Besides, proscribed experiments will need continued re-evaluation in the light of new techniques and new understandings, and it may be that research done in forbidden territory, however lacking in approval by a majority of scientists, positively helps in reinforcing or easing the moratorium.

We have a broad general sympathy with the aims of the NAS group and we recognise in their statement important ground-breaking in the scientific community. Nevertheless, we would not at present wish to commit ourselves to any blanket policy of turning away reports from those who work on regardless. The issue of publication, though, certainly merits an airing at next February's international meeting.

A hundred years ago



While progress has been made with gigantic strides in many directions, in engineering and in mechanics generally; while railways, steamboats, and electric telegraphs have extended their wonders to the most distant parts of the world; and while trade, with these aids, is bringing to our shores the produce even of the most distant places, to add to our comforts and our luxuries; yet, when we come to look to our homes, to the places where most of our population have to spend nearly the whole of their lives, I think we must find, with regret, that, in matters pertaining to the salubrity and general amenities of our towns and houses, as places for residence, due progress in improvement has not been made. Our house drainage arrangements are habitually disgracefully bad; and this I proclaim emphatically, alike in reference to the houses of the rich and the poor. We have got, since the early part of the present century, the benefit of the light of gas in our apartments; but we allow the pernicious products of combustion to gather in large quantities in the air we have to breathe; and in winter evenings we live with our heads in heated and vitiated air, while our feet are ventilated with a current of fresh, cold air, gliding along the floor towards the fireplace to be drawn uselessly up the chimney. A very few people have commenced to provide chimneys or flues to carry away the fumes of their more important gaslights, in like manner as we have chimneys for our ordinary fires. In mentioning this, however, as a suggestion of the course in which improvement ought to advance, I feel bound to offer a few words of caution against the introduction of flue pipes for the gas flames rashly, in such ways as to bring danger of their setting fire to the house.

From the Presidential Address of Prof James Thompson to the Mechanical Science Section of the British Association, in *Nature*, 10, 393, Sept 10, 1874.





Senator Kennedy: inspired anti-foetal research bill.

Guinea-pig charters

A number of ethically questionable research practices brought to light recently in the United States have led to calls for tighter control of human experimentation. Colin Norman discusses some of the regulations which have been proposed and their effect on research.

MANY American scientists hoping to conduct experiments on their fellow men and women will soon have to submit the ethics of their research to intense scrutiny by a raft of committees before they can get any funds. Some will then find people constantly looking over their shoulders while they carry out their experiments, others will find whole avenues of research shut off by the federal government or by state and local laws. In short, the traditional freedom of the scientist to organise his work is about to see drastic changes.

Human experimentation, like presidential power, has recently been a subject of national debate thanks to some widely publicised examples of ethical bankruptcy. Such examples include allegations that a number of poor black men who were known to have syphilis were left untreated so that the course of their disease could be monitored, that prisoners have been subjected to painful and essentially pointless medical experiments and that several children have been left mentally maimed by psychosurgery. The allegations have led to strident demands for more control over human experimentation, and the vocal anti-abortion lobby in the United States has also left its mark in the area of foetal research.

The controls have come from four directions: Congress, the Department of Health, Education and Welfare (HEW), state and local governments,

and the courts.

At the Congressional level, a bill has been passed and signed into law by former President Nixon which sets up an eleven-member national commission on human experimentation whose duty will be to draft a set of regulations to protect human subjects of biomedical and behavioural research. The commission, which should be established later this month, has been given two years to complete the task and has been asked to concentrate its attention on areas such as research with children, prisoners and the mentally disabled, where ethical and legal problems abound.

As for research on pregnant women and foetuses, the bill—a measure inspired by Senator Edward Kennedy called the National Research Act—imposes a four-month ban on federal support for research involving foetuses with beating hearts, before or after an induced abortion. While the ban is in effect, the commission will draw up regulations governing such research.

Meanwhile, HEW has been moving independently to strengthen controls over human experimentation supported by the National Institute of Health (NIH), the National Institute of Mental Health (NIMH), the Food and Drug Administration (FDA) and other HEW agencies.

The department has already imposed a blanket provision that human experimentation will only be supported at institutions which have established review committees to scrutinise the ethics of individual studies before application is made for funds, and it is now in the middle of devising extra protection for children, pregnant women, foetuses, prisoners and mental patients. Last week, HEW proposed some tough regulations in that regard.

At the state and local level, several laws have been passed which impose restrictions on foetal research, the most prominent being a recent move in Massachusetts to outlaw research on living foetuses, before or after abortion. While the federal regulations would simply cut off funding for such research, the state laws would make some types of foetal research a crime punishable by a stretch in jail.

As for the courts, the whole concept of legal consent to take part in research as it applies to children and mental patients is under challenge in at least two cases, and in Boston four doctors are now awaiting trial on charges arising from some foetal research they conducted in 1971. Thus, as is common practice in the United States, the courts are likely to define some of the parameters of social policy.

All these attempts to regulate the practice of human experimentation involve a delicate balancing act by weighing potential benefits to society from medical research against the possible risks to those who take part. It is a balancing act which has its roots in the preamble to the United States Constitution, which states that the Constitution is designed to “promote the general Welfare, and to secure the Blessings of Liberty”.

According to Dr Donald T. Chalkley, chief of NIH's Institutional Relations Branch, which has been drafting the HEW regulations, the real problem is that “we have a public concept of research that is based on fiction—on Clockwork Orange or Lord of the Flies experiments—but 90% of the research involves no more than the taking of a blood sample”. This public concept is causing legislators to pass unduly restrictive laws, such as the blanket ban on foetal research.

Although stopping well short of imposing a flat ban on research involving living foetuses, HEW's proposed regulations would severely restrict such studies, and outlaw some kinds of research entirely. In short, such experiments would be subjected to three separate ethical checks, and they would have to be confined to a very narrow range of experiments in order to qualify for HEW support.

Ruled out would be a wide range of experiments on foetuses *in utero*, before they are aborted. Such prohibitions are also embodied in the Massachusetts foetal research law, and when that law was being debated, many scientists warned that the effect would be to stop a number of valuable research projects, and possibly to place future generations of children at risk.

The prohibition would prevent drugs or vaccines being administered to pregnant women about to undergo abortions in order to see whether they cross the placenta into the foetus. For some substances, such as rubella vaccine, such knowledge is essential because of the dangers that foetal abnormalities may be caused if the substance does cross the placenta. Dr Chalkley acknowledged in an interview last week that banning such research could place children at risk, but he pointed out that the problem is to balance the individual rights of the foetus and its mother against the potential promotion of the general welfare of children, both of which are equally protected by the Constitution.

HEW also chose to draw the line against foetal research *in utero*, which is not designed to benefit the mother or that particular foetus, because if the foetus is placed at risk by such experiments, it would be very difficult for the mother to change her mind about going through with the abortion.

It is worth pointing out, however,

that although the HEW proposals would permit experiments designed to benefit the mother or the foetus, they would rule out control experiments because they ban all non-therapeutic studies on living foetuses.

If the setting of regulations governing foetal research is fraught with difficulty, the situation regarding research on children is even more difficult. The benefits from paediatric research are obvious, but the ethical and legal problems are also legion, and HEW, which has been trying to set some regulations in this area for the past couple of years has so far been unable to do so.

The problem is essentially two-fold. First, there is the difficulty of defining what kinds of research should be permitted and what should be outlawed. As Chalkley put it last week, if you shut off an area of research now because the potential benefits are outweighed by the risks, you may be opening the door to new diseases. And second, there is the problem of getting informed consent from minors.

Can a parent or guardian legally and morally consent to have his or her child take part in a research project which might involve some risk, but which will not benefit the child directly? A law suit, filed against the University of California by an attorney, James R. Nielsen, contends that no "invasive" medical research procedures should be practised on a normal healthy child unless they hold potential direct benefit for the child.

Again, if research directly beneficial to the child is allowed but every other type of paediatric research is outlawed, one effect would be to prevent the use of control groups in any such experiment. Clearly, the issue of human experimentation is far from dead, and verbal battles will be fought on a variety of fronts. But at present the course of events at the federal level is far from clear.

The most likely scenario, is that HEW (open for comments until November) will put its regulations into effect next year, and the commission will suggest amendments in 1976. □

LAST month, the US Department of Health, Education and Welfare (HEW) proposed some tough regulations governing research on prisoners, mental patients, pregnant women and foetuses. Designed to protect the rights of those being experimented upon, the regulations would establish a number of ethical checks on research projects, and even ban some types of experiments entirely.

First, under regulations already in effect, every institution in receipt of funds from HEW must establish an Organisational Review Committee, composed of people from a variety of occupations, to review research protocols involving human experimentation before they are submitted to HEW for funding. This first ethical check will weigh the benefits to be derived from the experiment against the risks to those taking part, and it will also ensure that adequate provision is made for obtaining informed consent from the subjects.

In addition, HEW is now proposing that another committee—the Consent Committee—be established at the institutional level to provide continuing ethical review of experiments involving prisoners, mental patients and pregnant women. This committee will oversee the actual selection of subjects, make sure that they consent to take part without coercion and after having been fully informed of the risks, and that they are able to opt out at any stage if they no longer want to take part. The new HEW proposals would also impose the following restrictions:

● By far the most controversial por-

tion of the proposed regulations deals with research on pregnant women and foetuses. First, HEW is suggesting that yet another ethical check be applied to such studies from an Ethical Advisory Board, to be established in every HEW agency funding foetal research.

These boards, which would consist of members appointed by the Secretary of HEW, from outside the government would review all grant applications for such studies after they have been approved by Organisational Review Committees.

All research involving foetuses *in utero* on pregnant women would, however, be banned unless (a) "the purpose of the activity is to benefit the particular foetus or to respond to the health needs of the mother", or (b) the research is aimed at "evaluating or improving methods of prenatal diagnosis, methods of prevention of premature birth, or methods of intervention to offset the effects of genetic abnormality or congenital injury", in which case, "the activity [must be] conducted as part of (but not prior to the commencement of) a procedure to terminate the pregnancy". The mother's consent would be required for such research, and the researcher should have no part in determining either the timing or method of abortion or the viability of the foetus.

As for research on abortuses, the proposed regulations would require that relevant animal studies first be completed, that the mother's (and if possible the father's) consent be given, and that the vital functions of the abortus are not artificially maintained. Further-

more, HEW is proposing that experimental procedures which would terminate the heartbeat or respiration of the foetus should be banned, but it stops short of calling for complete cessation of studies on foetuses with beating hearts.

● As far as research on prisoners is concerned, HEW suggests that an Organisation Review Committee and a Consent Committee should provide adequate protection for research subjects, by making sure that prisoners are offered no special inducements or threats to make them take part. A preamble to the regulations specifically rejects the notion that since prisoners have little freedom of choice in anything, they are incapable of freely giving their consent to take part in research projects.

● Involuntarily detained mental patients pose a slightly more difficult problem. The regulations, however, would permit research on such people provided that the study is "related to the etiology, pathogenesis, prevention, diagnosis, or treatment of mental disability or the management, training, or rehabilitation of the mentally disabled and seeks information which cannot be obtained from subjects who are not institutionalised mentally disabled".

In other words, HEW is attempting to rule out the use of mental patients for studies unrelated to their mental illness.

The proposed regulations are available from the Institutional Relations Branch, Division of Research Grants, NIH, Bethesda, Md. 20014, USA.

international news

OPENING this year's British Association conference at Stirling, the President, Sir John Kendrew, called for changing attitudes amongst scientists to counteract the growing indifference and even hostility towards science shown by the non-scientific public.

A note of gloom pervaded the first few minutes of the address, as Sir John outlined the problems facing the practice of science today. The relationship of science with society has become slightly defensive on the part of the scientists. Increasing financial restraint on scientific research and the downgrading of scientific adviser posts both in Britain and the United States have not helped matters.

The hostility towards science arose because people saw it as the handmaiden of economic growth, as the creator of pollution, as a support for the military and as a source of little understood dangers such as genetic engineering and computer data banks, or even as a negation of humanity itself.

But, said Sir John, changing to a more optimistic note, the scientists' feelings of pessimism and guilt are unjustified. Science has proved itself in the past and there will be an absolute need for science in the future. But hostile critics will only be converted by a change in scientists' attitudes. Scientists must focus on the challenges which will face us in the future and be less despondent about some of the immediate practical difficulties.

The main challenge was to decrease the widening gap between our standards of living and those of the poorer peoples. Whether we favour a "growth" or a "no-growth" society said Sir John, we could not be content that a no-growth society should involve most of the world's population living in undernourishment, squalor and disease.

The concept that the pursuit of knowledge as an absolute good in itself was the prime motive of scientific research needed to be re-examined, said Sir John. Although a good motive, it had not been, nor ever could be the only one. After the War, in which many scientists had applied their skills, there had been a reaction towards pure research for its own sake, he said. But the world had changed since the 1950s and the challenges had changed, too. "In my view," said Sir John, "it is neither socially productive nor intel-

B.A.—first the bad news

lectually satisfying to refuse to modify the old attitudes or to respond to the new challenges." Much contemporary research might seem to future historians like the minute and pettifogging scholasticism of mediaeval times.

Even the increasingly tight research budgets might compel scientists to direct research into fields that may actually turn out to be more productive than those that could be afforded in times of plenty. Limited budgets could also be optimised through international cooperation, despite the pitfalls of bureaucracy, politics and commercial restraint.

Sir John went on to discuss specifically the problems arising from the genetic manipulation of bacteria, an example of a scientific advance which could hold dangers for the human race and which has recently been much debated in public.

In these experiments, pieces from animals or viruses can be linked with bacterial DNA in such a way that the complete DNA molecule can replicate inside a bacterium. It was feared that the deliberate or accidental introduction of cancer-causing genes into bacteria for further study—an admirable end in itself—could lead to these genes becoming widely disseminated in the human and animal population, since the organism most widely used for this type of work was the common gut bacterium, *Escherichia coli*. But the incorporation of certain animal genes making useful products could be of great benefit. No-one yet knew or could foresee, all the possible dangers as these techniques were still relatively untried.

Sir John drew an analogy with the state of nuclear physics at the beginning of the last War, when it became clear that nuclear fission could make possible the development of new power sources but could also be used to construct an atomic bomb.

He approved the action of some American scientists and the British Medical Research Council in calling for a voluntary moratorium until the dangers of this work had been evaluated. A permanent international body made up of molecular biologists them-

selves, should recommend what types of experiments should or should not be carried out and under what conditions of security. Also a fact-finding programme of research in this area should be conducted under conditions of high security and the results published fully in the open scientific literature.

This was a case, he said, where scientists must be seen to put their own house in order, or else others would step in and do it for them perhaps in ways which would lead to quite undesirable restrictions on what they do.

Even when decisions passed to the social or political forum, it was absolutely necessary that there should be effective communication of scientific knowledge upon which decisions could be based. Communication of science to the general public seemed to be failing at present and scientists should take trouble to translate their ideas and discoveries into language which anyone could understand. □

Science is fun

ON Saturday, August 31, Westfield College of the University of London held a carnival of science which it dubbed 'WES-POP'. The idea for this feast of fun grew out of a similar but larger event held at Aix-en-Provence last year. That event—AIX-POP—was arranged to coincide with a gathering of scientists from many nations for a conference on elementary particles. In spite of some initial scepticism among the physicists cajoled into cooperating, AIX-POP proved a roaring success, in that hundreds of ordinary citizens were brought into contact with the scientists in small groups, where the scientists were forced to communicate intelligibly at a personal level.

Professor Elliot Leader, of Westfield College, was one of the participants in the Aix event, and was sufficiently enthusiastic about the idea to organise something along the same lines in north-west London. The event was widely publicised locally by means of posters in fairground style offering such treats as "See your friends bombarded by cosmic rays!" and "Watch the amazing lasers in action". And further publicity was lavishly provided by the local newspaper, which allowed Professor Leader space for three feature articles about physics and the festival—that in itself is remarkable enough for any British newspaper. □



PROFESSOR F. Graham Smith, presently Professor of Radio Astronomy at the University of Manchester, is to succeed Dr Alan Hunter as Director of the Royal Greenwich Observatory (RGO) at Herstmonceux, Sussex. Stated that baldly, the appointment might cause consternation—what could be in the minds of the members of the Science Research Council (SRC) that they should appoint a radio astronomer to head England's principal optical observatory? But this superficially bizarre decision will not seem so odd to anyone who has followed the recent development of astronomy in Britain, nor to anyone who is familiar with Professor Smith's career; indeed, this could be one of the best things to happen to optical astronomy in Britain for a long time.

The situation has been confused, of course, by Professor Margaret Burbidge's brief yet stormy reign as Director of the RGO. On her resignation the then Deputy Director, Dr Hunter, took over the top job. But that could only be an interim appointment, since Dr Hunter reaches retiring age at the end of 1975, after 38 years with the observatory.

Now that situation is resolved. Professor Smith (pictured above at Jodrell Bank) will join the RGO staff this autumn, and will serve as deputy to Dr Hunter until the end of 1975. This should be ample time for him to learn the ropes, if past experience is anything to go by.

New director for the RGO

by John Gribbin

Professor Smith is far from being a narrow specialist in radio astronomical matters. Now 41, he completed the bulk of his formal education before radio astronomy developed as a discipline in its own right, and his PhD (awarded in 1952) must have been one of the very first awarded by the University of Cambridge for radio astronomy work. Following spells at the Telecommunications Research Establishment, Malvern, and at the Cavendish, he moved to his present post in Manchester in 1964. There can be no doubt that his influence is in no small measure responsible for the fact that Manchester is now recognised as a place where first class theoretical work in the field is done, as well as being the home of one of the world's leading radio astronomy observatories. In particular, Professor Smith has recently worked on theories of the emission mechanism of pulsars, cosmic rays and galactic radio sources.

In 1969, Professor Smith gained something of a reputation as a trouble-shooter for the SRC, when he spent 10 months as Deputy Director of the Radio and Space Research Station (now the Appleton Laboratory) to assist with

a review of the station's research policy. So Professor Smith takes with him to Herstmonceux a thorough knowledge of the workings of the SRC machinery and a great deal of administrative experience. There seems no likelihood that this appointment will fail either through personal conflicts between the Director and the SRC or through any lack of ability, and the RGO can thus look forward to a prolonged period of stable development. It is too early yet to see what direction that development will take, although the fact of stability alone will be welcome enough after recent troubles. But Professor Smith told *Nature* that he was particularly anxious to move to the RGO at once, rather than wait until 1975, in order to be fully involved with developing the proposed Northern Hemisphere Observatory. He emphasised that this is "a marvellous opportunity" to get to grips with the engineering side of optical astronomy, and to meet the people involved on a working basis, before he disappears into an office labelled 'Director'.

After much prevarication, that Northern Hemisphere Observatory now at last looks like becoming a reality. With Professor Smith playing an important role, and with the SRC's infrared telescope now definitely intended for a Hawaiian home, it begins to look as if there really will be a Northern Hemisphere Observatory, almost certainly in Hawaii, in the not too distant future. □

The case of the cancelled hovertrain

by Angela Croome

THE case of the British Government-funded experimental development of the hovertrain on which a White Paper (Tracked Hovercraft Ltd, Cmnd 5711) has just been issued is a classical example of how not to manage an innovative high-technology assessment project. What is so especially discouraging is that the sequence to cancellation could be predicted five years—and £5½ million—earlier with very considerable reliability. More must have been written and minuted in Britain on the subject of fostering and managing technological innovation than almost any other technological subject since the last World War. Yet no lessons seem to have been learnt; no guidelines derived.

The White Paper has been produced under pressure from Parliament's Select Committee on Science and Technology which held an inquiry which took up much of the first half of 1973 and some 500 pages of reports, into the circumstances surrounding the close-down of Tracked Hovercraft Ltd. and its full-scale vehicle test track near Cambridge. The summary Report (July 24, 1973) of the Select Committee was very critical of almost every aspect of the business—of the cancellation of the project no less than the manner in which it was done (the bulk of the 150 staff were given less than 24 hours to be out of their offices, for example); of the Government's role, of the way innovation is managed (or mismanaged) in Britain and the adequacy of the National Research Development Corporation for this task. It specifically proposed that there should be a 'high-level' review of the organisation needed to develop innovation and asked that the Government look again at the 1972 Select Committee recommendation of a small expert Department headed by a Cabinet Minister to look after Government sponsored R and D; further, that the THL test track at Earith should not be scrapped but become a multiuser research facility run by a consortium of British universities with interests in novel surface transport and headed by Imperial College.

The White Paper in response to this swingeing assault can only be described as a mouse. It dodges out of dealing with the main criticisms by attributing them to a previous Government for which it was not responsible. Apart from enunciating the principle that "no promising technology ought to be stifled in infancy for lack of funds" it expresses entire satisfaction "that departmental organisations, supplemented



Professor Bhulchandra Jayavant demonstrates the principle of the Sussex magnetic train: all right for Brighton front, but how good at speed?

by the NRDC, provide adequate machinery for Government participation in the development and exploitation of inventions". As to providing some funds for maintaining the Earith test track as a multi-user facility, it denies the need for such a centre and by withdrawing restrictions on dismantling it laid down last year invites the speedy scrapping of the track. In the overall field of high speed public transport "the Government considers that the present effort and expense are appropriate in scale and pace".

The present effort and expense in this field amount to this. There is the multi-million pound British Rail development of the Advanced Passenger Train (APT) envisaged as running at up to 250 mph and coming into service on existing rail tracks in the 1980s. The development of the linear induction motor for fast tracked vehicle propulsions which THL at Cambridge had been fostering through its links with Professor Eric Laithwaite at Imperial College has been passed to the Brush subsidiary of Hawker Siddeley with a £400,000 Government grant against an investment of £100,000 by the company which it has been remarked has never made a linear motor before. The Wolfson Foundation has put about a quarter of a million into research into mass transport vehicles employing magnetic propulsion and/or levitation, notably at Sussex and Warwick universities. Now the Science Research Council is putting up some funds for similar work in the universities. Two things may be said about this pattern of effort. None of the work—save possibly some of that in the universities—looks beyond 1985, the date which in

his much-criticised evidence to the Select Committee the previous Transport Minister (Mr Peyton) considered his departmental limit. Secondly, coordination of these activities now involves three ministries and the public sector. The dangers of fragmentation of effort have been reiterated continually during the debate on the organisation of government R and D no less than over the Tracked Hovercraft inquiry. If communication and coordination was inadequate when only British Rail's APT project and NRDC's THL were involved as the Select Committee indicated, how much worse is it going to be now?

A glance at the scale and pace of effort in other countries provides some sharp contrasts. Japan has perhaps the largest programme in high speed mass transport development. It is costed in thousands of millions, very long-term, much interested in electro-magnetic levitation employing cryogenics and no less than 60 professors are said to be involved full time. The United States has in August taken the opposite step taken by the British Government last year by dropping further work on its high speed passenger train (called the Improved Passenger Train Project) and switching the \$5.6 million involved into support of tracked hovercraft transport. A huge test site is under construction in the Colorado desert where five different types of trackway amounting to some 70 miles altogether are being laid out as the High Speed Ground Test Center to test types of high speed propulsion including linear motors and suspension including both air cushion and mag-lev (magnetic levitation); as well as alternative modes

TOPICS of public concern arising from recent medical advances are given an airing in the British Association's most recent publication, *Social Concern and Biological Advances* (British Association, 50p).

A wide-ranging committee of scientists, lawyers, Members of Parliament, theologians and others was set up in 1972 to investigate the social, ethical and legal implications of certain medical advances, notably those in the fields of *in vitro* fertilisation and genetic manipulation.

Discussing artificial insemination by donor (AID) the committee consider that the legal status of any child conceived through AID needs to be properly defined as such children now occupy a twilight sub-legal zone. The genetic makeup of the donor is another important issue, and the committee call for a decision as to whether prospective sperm donors should be examined medically and as far as it is possible, genetically. The control of sperm storage banks is another problem of some concern. The government should decide immediately whether private banks are to be allowed and standards and supervision for such banks should be defined.

The committee recommends that research on *in vitro* fertilisation should continue as its possible advantages outweigh potential disadvantages. But there is the potential for abuse, and so the medical profession and government should keep a careful eye on new developments in this field and should be prepared to act quickly to prevent abuse.

Genetic screening for some genetic-

ally determined crippling diseases is now relatively common. Although this is superficially of great potential for reducing the numbers of children born with severe mental and physical abnormalities, the moral problems at both the social and personal levels can cause acute distress.

Social attitudes towards abortion can influence both the doctor's recommendation and the mother's response

Biomedical advances give rise to concern

when an abnormality is detected especially in borderline cases where the degree of the abnormality cannot be predicted precisely. Less obvious but equally distressing, is the risk that a woman carrying an affected foetus could be subjected to social censure if she refused to be screened or undergo an abortion. This censure could also carry over to the child itself.

Under the broad heading of genetic engineering, eugenics and pre-natal sexing, the committee considers gene therapy, potentially useful but still experimental; sterilisation of people carrying defective genes; and the possibility of being able to choose the sex of one's child.

A policy of selective sterilisation needs to be scrutinised particularly carefully, as individual rights must be protected and the definition of 'normality' can be open to abuse by political and social systems which seek to manipulate it for their own ends. The committee make no specific recommendations on this head but advise

continuing review of developments in this field.

The committee seem undecided as to whether pre-natal sexing will have much appeal for Western societies but recommend that official bodies should be prepared to act quickly to prevent precipitous and unadvised applications of new sex selection procedures.

The report also covers the chief moral problems involved in organ transplantation such as defining the moment of death and the ownership of the dead body. "Any definition of death" it says, "implies an understanding of what constitutes life".

The 1961 Human Tissues Act covers the transplantation of organs but is unsatisfactory on some points. Under this Act the legal possessor of the dead body can authorise the removal of the organs. But who, asks the committee, is the legal possessor, and what constitutes a reasonable inquiry to gain permission from relatives to remove organs for transplantation? Also, should Britain operate a "contracting in" or a "contracting out" system whereby people indicate whether or not they wish their organs to be used after death?

The committee favour adopting some of the recommendations of the 1969 McLennan Committee. These are that the present system should stand but that it needs legal reinterpretation to remove ambiguities and to facilitate the supply of organs to transplantation. Registers should be kept both of those who wish to contract out, and on the other side of the coin, those who specifically wish to donate organs after their death should also be registered.

of urban transport.

In Germany 45 miles of test track are now planned at a site near Augsburg and very large public sums (£100m for R and D alone) have been invested in high speed transport for the future with particular emphasis going to linear motor development and magnetic levitation. Krauss Maffei which won the contract for the Toronto project at about the period that the Government announced the cancellation of the tracked hovercraft has switched its design from the 'conventional' double-sided linear motor to the single-sided type first conceived by Professor Laithwaite and 'proved' on the THL vehicle which Krauss Maffei had visited.

In the light of this amount of world activity and interest the ostensible reason for last year's Government cancellation of the tracked hovercraft, that there was no customer or market for it, seems extraordinarily thin. In fact some of the by-products of THL—the single-sided linear induction

motor, the box-beam track for instance—have, as described, been adopted in overseas projects. The Select Committee accepted that Britain had about a five-year lead in overall hovertrain technology—and this was being reflected in consultancy fees particularly in North America. Professor Laithwaite's even more recent breakthrough which he calls the 'magnetic river' and which offers propulsion and controlled suspension in three axes from the same transverse-flux linear motor running on normal mains AC seems to put British research in the field of high speed transport even further ahead. Professor Laithwaite claims that he will have a production prototype of this double-purpose motor within six months, and it is particularly suitable for speeds of over 250 mph (where British Rail's APT runs out). Similar claims for the University of Sussex electro-magnetic attraction system, recently demonstrated in an urban passenger carrying version being

developed for a 2½-mile shuttle along Brighton front, seem likely to be misplaced. Over 50 mph, a serious drag, develops from eddy-currents set up in the track (a problem that Krauss Maffei are encountering in the Toronto project now). One is forced to believe that the only customers that the British Government's chief scientist departments accept are home customers and that in the case of the tracked hovercraft it was especially unfortunate that the customer department DoE was also the sponsoring department of British Rail and its pet the APT. Customers beyond 1985 are not being considered and there seems no mechanism for considering the general public as a customer.

The Government has turned down the Imperial College form of proposal for keeping the Earith test track in being. Several practical problems now arise. The estimated cost of dismantling the track is £200,000 and THL has no funds left so the Government will

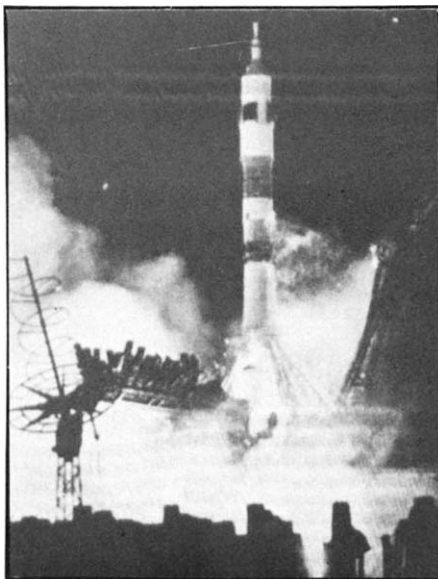
THE brief flight of Soyuz-15 (August 26-28) has led to considerable speculation as to some possible failure which may have led to its curtailment. The report of a naked-eye sighting of Soyuz-15, which at 4.30 a.m. (GMT) on August 28 was seen to have overtaken the orbiting space-station Salyut-3, suggested that something had gone amiss, preventing an intended docking of the two craft.

In fact, however, the official *Tass* reports do little to substantiate these speculations. Although the first statement does mention Salyut-3 it does so only in an oblique manner, stating that: "The purpose of the launch of Soyuz-15 is the continuation of the scientific research and experiments in outer space begun on July 3 of this year in the flight of the transport ship Soyuz-14 and the station Salyut-3". The mention of Salyut-3 certainly suggested that the two craft would, in some way, participate in a joint experiment; the next *Tass* release, however, stated clearly that this would not be a link-up. The experiments were intended "to work out techniques of piloting the craft in different regimes of flight. In the process of manoeuvring the craft, Soyuz-15 made a number of approaches to the Salyut-3 station. The cosmonauts controlled the operation of all systems of the ship, carrying out observations of the stages of approach to the station". Soyuz-15, the release continued, was now preparing to return to earth.

The *Tass* account of the descent stressed the details of the flight procedure—the use of the braking motors to align the craft before descent, the release of the parachute system and the firing of the braking motors immediately before touchdown. The aim of this last stage of the experiment was to test "methods and means of finding and evacuating a spaceship

Soyuz – 15: didn't fall, wasn't pushed

from Vera Rich



Soyuz-15 blast-off: AP wire picture.

which had landed in night conditions". This was the first true night landing, although Soyuz-10 landed in predawn conditions. The search and recovery stage of Soviet space ventures has always held its own special hazards—one early pair of cosmonauts had to fight their way back to civilisation through the snow for two days. Nevertheless, according to V. A. Shatanov, the commander of Soyuz-10, the need to practise night-landing is becoming more and more necessary, since the necessity to land at a given time of day imposes rigid conditions on the flight-plan even before take-off.

Tass reports are traditionally brief and uncommunicative, frustrating the desire for further information with

such phrases as "according to its assigned programme", or "carried out its aims", without specifying full details of that programme or those aims. They are in a style therefore admirably suited to effect a cover-up of a partially aborted programme. But what if the speculations are wrong, and there was no break-down, and Soyuz-15 was planned from the beginning as a two-day mission. The need to practise night-landing, thereby eliminating rigidity of the flight plan, fits in well with the plans for the joint Soyuz-Apollo programme planned for 1975. (There could be considerable loss of face if either party had to ask for a revision of the joint flight-plan because its crew and ground support could not manage a landing under such-and-such conditions!) The practice of docking approaches from various positions, and the insistence that the cosmonauts were in total control of the craft is also interesting. There has always been a tendency in the Soviet manned space programme to incorporate a certain amount of ground control — the early cosmonauts were little more than passengers in the craft that carried them.

Speculation has suggested some technical failure in the Soyuz-15 craft, with possible setbacks to the Soyuz-Apollo programme. This may be the true explanation—but there remains the possibility that the *Tass* statements, do give, albeit briefly, the real objectives of the mission; that the flight of Soyuz-15 was intended simply to test out tricky manoeuvres, so that, in the joint mission, Soviet prestige might be duly maintained, by Soviet cosmonauts able to perform their part of the mission even in adverse circumstances—making docking approaches from any position, and able to cope with a touchdown at any hour of the day or night.

have to find some kind of subvention to enable the company to remove the one mile existing track. There are two further miles of track in the form of unassembled beams in store at the manufacturers. What is to be done with them? With THL in nominal existence only, how are the numerous patents and the accumulated expertise to be exploited? It is impossible that the last has been heard of the tracked hovercraft and its company. Professor Laithwaite and his magnetic river are probably its greatest 'liquid' asset. He has not been heard from since the White Paper's publication but it is understood that alternative support is under discussion for the track and the plan to make it part of a possible joint European rapid transit centre.

There is a meeting of the EEC inter-city working party in October in which ex-THL staff take part. The Select Committee may be regretting the move which enabled the Government to publish its meagre response while Parliament was in recess. This does not reflect on the excellence and thoroughness of the Committee's original Report and inquiry and there must be every expectation that it will either call for more evidence or make its views known through a debate on the White Paper as soon as Parliament is reconvened possibly accompanied by a further publication.

As to the sorry tale of the hover-train's relevance for the bigger issue of the proper support of innovation today, Sir Christopher Cockerell, in-

ventor of the parent concept, the hovercraft, gives the best summary (in a Memorandum to the Select Committee):

"To carry through a major development, a powerful lobby is required — example, Concorde; or a very powerful position — example British Rail and the high speed train; or prestige and many jobs at stake—example, Rolls Royce and the RB 211. . . . Now consider a brand new idea. It starts with neither money, facilities, a proven performance, a proven market, a lobby, prestige, jobs at stake, nor even people who understand it. . . . What is missing for big projects is a suitable machinery to cover the stages between NRDC's limited support and the fully commercial stage".

news and views

Development of hominid locomotion

THE uniqueness of the human locomotor pattern has fascinated students of evolution for a long time and the past few years have seen an important increase in the complexity, detail and scope of locomotor studies. Erect bipedalism is, of course, not found solely in man, but it is a rare form of locomotion in the animal world and both the reasons for its development and the nature of that development itself have stimulated much research.

Although there is no consensus regarding why erect bipedalism has characterised the human lineage for at least several millions of years, there seems to be some agreement that no single factor 'caused' bipedality. In other words, a combination of environmental and behavioural situations, combined with genetic changes, probably worked together in a positive feedback relationship. For example, changes in the environment and behaviour might have provided new selective backgrounds favouring the incorporation of genetic advances in manual flexibility and dexterity, cerebral complexity and bipedality. Changes of such physiological and anatomical nature could, in turn, provide new bases for further behavioural developments. In this way, the development of bipedality is not viewed as an isolated event but rather as just one facet of highly interrelated and even interdependent evolutionary processes. This approach to mutual causality in processes of social, cultural and physical development has come to be called "the second cybernetics" (Maruyama, *Am. Sci.* **51**, 164-179; 1963). Such reasoning is an important advance over earlier chicken-egg hypotheses in that it delineates the evolutionary process as taking place on several levels simultaneously. A logical extension of this point of view is to suggest that it is the interrelationships between the levels which are in fact evolving and not the levels themselves.

Most of the recent studies on human locomotion, however, have been more concerned with analysis of its physical development and biochemical characteristics. Many different types of investigations have been used; anatomy, biomechanics, radiography, cineradiography, electromyography, as well as multivariate statistical techniques, have all been focused on the evaluation of locomotor patterns in living primates (including man) and some of this work has been used to reconstruct locomotor patterns in fossil groups (see Oxnard, *Syst. Zool.* **22**, 409-424; 1973 for a recent review). One result of the palaeontological investigations has been that despite the rarity of erect bipedalism in the animal world probably two slightly different forms of this locomotor pattern have been present, apparently simultaneously, within the human lineage. The two patterns are contemporary at Lake Rudolf possibly as early as 3 million years ago and this suggests a profound and early dichotomy within the Hominidae. Good evidence of this separation exists in the skull and dentition (Leakey, *Nature*, **237**, 264; 1972; Day and Leakey, *Am. J. phys. Anthrop.*, **39**, 341-354; 1973) and it is also demonstrated in the postcranium, most clearly in the femur, talus and tibia.

In the human fossil record the femur is the best represented bone of the postcranial skeleton and in the relatively large number of femora now available two distinct patterns seem to have emerged. One of these, the 'australopithecine' pattern, shows a small femoral head with a long flattened neck, non-flaring greater trochanter and a high degree of shaft obliquity or tilt (Walker, *J. hum. Evol.* **2**, 545-555; 1973). Material showing this pattern is known from South Africa (SK82 and 97), Olduvai Gorge (OH20) (Day, *Symp. Zool. Soc. London.*, No. 33, 29; 1973) and East Rudolf (KNM-ER 738, 815, 736 and 1505, Walker *op cit.*). Moreover, radiographic evidence of the internal structure shows some possible distinctions in a greater thickness of the shaft walls and in the orientation and distribution of the internal trabecular structures (Walker, *ibid.*). Within the Lake Rudolf material a second morphological complex has been identified and this pattern conforms more closely with that seen in living man: a larger femoral head, shorter and rounder neck and flaring greater trochanter are the most visible similarities. KNM-ER 737 (Day and Leakey, *Am. J. phys. Anthrop.*, **39**, 341; 1973) and KNM-ER 1481 and 1473 (Leakey, *Nature*, **242**, 447; 1973) show this pattern and have, in fact, been attributed to the genus *Homo*. Further evidence of the dichotomy may also exist within the talus. The talus is an important bone for locomotor assessment since it acts as a hinge joint between the lower leg and the foot and in this position can potentially reveal much about the structure and function of the foot. Though the talar specimens TM 1517 (from Kromdraai), Olduvai Hominid 8, and KNM-ER 1476 and 1500, show some interesting variations within the group, they seem to form one morphological pattern. TM 1517 and OH 8 have been intensively evaluated through anatomical and multivariate techniques (Day and Wood, *Man*, **3**, 440; 1968) and through multivariate techniques alone (Oxnard, 1973); OH 8 has been subjected additionally, to biomechanical analysis (Preuschoft, *Folia Primatol.* **14**, 209-240; 1971). Although there is agreement that the two specimens share many features they may have differed somewhat in the orientation and functional capabilities of the great toe. The suggestion of the Kromdraai talus is that the great toe was somewhat divergent (Le Gros Clark, *J. Anat. Lond.* **81**, 300; 1947) and this may have resulted in a more flexible foot capable of "some climbing ability" (Robinson, *Early Hominid Posture and Locomotion*, University of Chicago Press, 1972) and some "grasping" ability (Le Gros Clark, *op cit.*). The OH 8 specimen (a nearly complete foot), has, however, a great toe which is not significantly divergent although Preuschoft (*op cit.*, page 213) has suggested that it may diverge slightly more than in the original reconstruction.

Other talar material, notably KNM-ER 803 (Leakey, 1972 *op cit.*) and KNM-ER 813 (Leakey, *ibid.*, and Wood, *J. Anat.*, **117**, 203-204; 1973) has been attributed to *Homo*. Although several fossil hominid tibiae are now available from East Africa, no comprehensive study on this material has yet been completed and therefore any conclusions must remain very tentative. Preliminary studies again suggest, however, the existence of two morphological, and presumably locomotive, patterns. Among this material the partial skeleton, KNM-ER 1500 (including a tibia) and KNM-ER 1476 have been allocated to *Australopithecus* (Leakey, *Nature*, **242**, 447; 1973). Conversely, the

partial skeleton, KNM-ER 803 (with a tibia) has been allocated to *Homo* (Day 1973, *op cit.*); KNM-ER 1481 seems also to fall with this group (Leakey, 1973, *op cit.*). The tibia and fibula from Bed I at Olduvai Gorge (OH 35) has been tentatively allocated to '*Homo habilis*' (Leakey, Tobias and Napier, *Nature*, **202**, 7; 1964) but in view of recent developments in the field (Leakey, *Nature*, **248**, 653; 1974) perhaps the criteria for this taxon should be re-evaluated.

Thus several lines of investigation firmly suggest that a locomotor dichotomy existed within the bipedal early Pleistocene hominids. Although the functional nature of this dichotomy is not yet entirely clear it may be that the striding gait of *Homo sapiens* was not present within the 'australopithecine' group (Day and Wood, *op cit.*). Moreover, although some workers in the field insist on the use of a broad spectrum of analytical tools, there is a tendency in the recent literature to de-emphasise the importance of such comprehensive anatomical biomechanical and radiographic studies and to concentrate heavily if not exclusively on multivariate statistical evaluations. The basic mechanism of this approach involves quantification of the specimen and then, in the case of fossil material, interpolation of a function of these measurements into a comparative matrix composed of measurements from living primate groups and perhaps other fossils. This somewhat mechanistic approach, although admittedly useful as part of a broader

study has important limitations when used as a total analytical approach. First, some significant features of bones do not lend themselves to quantification. The disposition, orientation and density of trabecular bone are such features; the development and orientation of muscular markings are others. Second, such a method assumes that the locomotor pattern of the fossil has a known analogue: such an assumption is unproven, especially with regard to the fossil Hominidae, since it now seems likely that the 'australopithecine' pattern was unique. The hazards of this rather narrow approach were well demonstrated in a recent paper by Oxnard *op cit.* On the basis of only eight parameters of the tali of TM 1517 and OH 8 Oxnard's results suggested that the australopithecine foot had "distinct morphological affinities with the genus *Pongo*"—an affinity clearly unlikely on the basis of the total morphological appearance of the nearly complete OH 8 foot.

In summary investigations of the development of locomotion within the hominid lineage provides one of the more active and fascinating areas of palaeoanthropology today. It is important, however, that such studies be as broadly based as possible and utilise the research tools, analytical methods and descriptive models of many disciplines and only in this way is constructive insight possible.

From a Correspondent

The Earth gets more complex

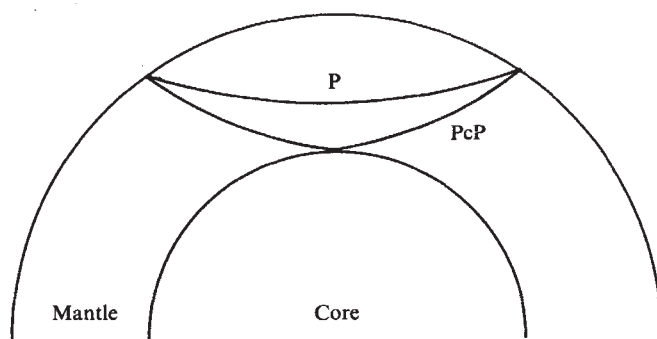
ONE of the major occupations of seismologists for many years has been to determine the travel time of compressional (P) waves and shear (S) waves through the deep interior of the Earth. A knowledge of travel time as a function of distance from source to receiver is essential in locating earthquakes—it is also a most important ingredient in determining physical parameters of the Earth. The more effort that has been expended, however, the further the goal has receded. First, it became obvious that there were strong lateral variations in elastic properties (of the order of 5%) in the upper mantle, so that individual sources and receivers needed appropriate corrections. Then in the past few years it has been increasingly clear that even below a depth of 700 km lateral variations persist. An article by Jordan and Lynn (*J. geophys. Res.*, **79**, 2679–85; 1974) highlights the advances now being made.

It is interesting that the first authors to show that the assumption of lateral heterogeneity at great depths was unjustified did not consider travel times at all. Travel times are relatively insensitive to the presence of inhomogeneities whereas the amplitude of the incoming signal and its direction of approach are much clearer indications of heterogeneity—although difficult quantities to invert for the purpose of making anything more than a qualitative statement. In 1969 some observations were made of amplitudes at source–receiver distances which were well into the range in which the core should have shadowed out P waves (Phinney and Alexander, *J. geophys. Res.*, **74**, 4967). It was clear that amplitude at a given distance was highly variable and depended entirely on which region of the core–mantle boundary was sampled in each particular instance. Geophysicists are loath to consider substantial lateral variations within the core (perhaps that will change too) so there were clear indications either that the core boundary had strong topography on it or that the physical properties of the rocks at the bottom of the mantle are laterally variable—even to the extent of 5%.

Then large arrays began to come into operation and

results from them posed some problems, in that the angle of approach predicted by classical methods was often up to 5° away from the measured angle of approach. This provoked much research from which there eventually emerged a feeling that the anomalies could not be properly explained without resort to marked lateral heterogeneity deep in the mantle (Davies and Sheppard, *Nature*, **239**, 318; 1972).

The time was ripe for another hard look at travel times and Julian and Sen Gupta (*Nature*, **242**, 443; 1973) came up with a very surprising result. When every care was taken to avoid lateral heterogeneity in the upper mantle by using only very deep quakes as sources, and when the data were (as far as possible) not bunched together in a computer, from which only a mean could be extracted, significant patterns emerged. By retaining a knowledge of the approximate path sampled by each data point, it was possible to see broad general regions in the deep mantle (mainly within a few hundred kilometres of the core–



Body-wave paths through the Earth's mantle.

mantle boundary) out of which P waves emerged up to 1 s early or late relative to a standard travel time table. Much of what in earlier studies had been regarded as general scatter arising from reading errors, crustal structure and so on was probably genuine evidence for deep lateral heterogeneity.

Seismologists, one suspects, accepted the travel-time results more readily than they had the rather more circumstantial evidence from amplitudes and arrays. In any case it now looked as if there was a whole new field of seismology opening up—mapping the deep mantle. Jordan and Lynn's results point to a clear and fruitful way to go about it.

On a seismic trace of a large earthquake many different signals can be identified and timed to a fraction of a second. In addition to the P and S waves, phases called PcP and ScS are clearly visible—they are reflections of P and S waves from the core-mantle boundary. If the time interval between the phases P and PcP is measured, it should, in a laterally homogeneous Earth, depend on source-receiver distance but not on anything else. Furthermore, any deviations in elastic properties in source or receiver region will be sampled almost equally by the two phases, which follow paths in the upper mantle that are relatively close to each other. Thus to a first approximation upper mantle anomalies are eliminated when the difference of times is taken.

What Jordan and Lynn find is that for an earthquake in South America recorded across the North American continent, the PcP—P and ScS—S times by no means conform to standard travel time tables. Along a certain azimuth in particular the differences are up to 1 s (PcP—P) and 4 s (ScS—S) away from the standard. This, in seismological terms, is a significant deviation. They go on to show that the anomaly most plausibly occurs along the path of the phases P and S, not the core-reflected phases, and so there is rather strong evidence for anomalous structure in a fairly narrow region at a depth of 600 to 1,400 km. It is thought that the P-wave velocity is at least 1% (and probably more) greater than that of the surrounding material.

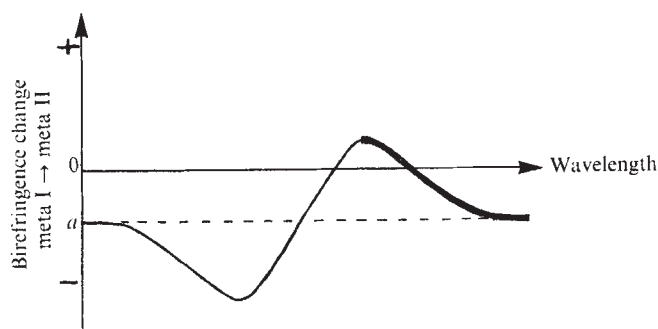
Is this just a strange quirk of nature? Another stamp to go in a collection which could expand rapidly? More than that, I think. Seismologists are often in danger of seeing their results as no more than information about seismic velocity in the Earth, but variation in velocity means variation in composition or temperature and that means a handle, however loose at present, on the geodynamics of the deep mantle. Do plates penetrate beyond 700 km? Is the deep mantle in convection? How laterally variable in temperature is the core at its boundary? The results of the past few years are beginning to make these questions just a little more reasonable to ask with some hope of an answer in the foreseeable future.

DAVID DAVIES

Birefringence and vision

IN the bleaching sequence of the visual pigment rhodopsin, the main spectral absorption shift (480→380 nm) occurs in the step between photochemical intermediates metarhodopsin I and II. This is observed in both intact rod outer segment membranes (Liebman, *Biophys. J.*, **2**, 161; 1962) and in detergent-solubilised preparations (Hubbard, Bownds and Yoshizawa, *Cold Spring Harb. Symp. quant. Biol.*, **30**, 310; 1965). This step may involve an unfolding or other conformational change of the opsin protein which could produce the primary physiological response in vision. There are several lines of evidence in support of this idea.

The Schiff's base bond attaching the retinal chromophore to the protein becomes chemically accessible at this step in solution (Bownds and Wald, *Nature*, **205**, 254; 1965), and probably in intact rods (Falk and Fatt, *J. Physiol.*, **183**, 211; 1966). There is also evidence for a conformational change in the protein on bleaching not specifically linked to the meta I→meta II step. Hubbard *et al.* (*ibid.*, 1965) inferred a conformational change in solution from ultraviolet absorption spectra. An increase in solubilised hydro-



dynamic volume has been observed by Heller (*Biochem. J.*, **7**, 2914; 1968). Circular dichroism measurements, which would be specific to protein conformational changes *in situ*, have indicated a change in solubilised pigment, but this change does not persist in the membrane (Shichi, *Expl Eye Res.*, **17**, 533; 1973).

In this issue of *Nature* (see page 31) Liebman, Jagger, Kaplan and Bargoot report a study on the conformational changes in rod outer segment membranes by optical methods similar to those used on excitable membranes. In the case of excitable membranes, there is a birefringence increase during the action potential (Cohen, *Physiol. Revs.*, **53**, 373; 1973) but in bleaching photoreceptor membranes there is a birefringence loss. What do these observations mean?

The refractive index of a material is the ratio of the speed of light in vacuum to the speed of light in the material, and a birefringent substance has different refractive indices for light polarised in different planes. In lipid bilayer membranes, where the hydrocarbon chains are aligned on average perpendicular to the plane of the membrane, the refractive index for the direction parallel to the chain axis is greater than that for the perpendicular direction. The birefringence, Δn , is the difference between the refractive indices for these two directions: $\Delta n = n_{\parallel} - n_{\perp}$. n and Δn are functions of the wavelength at which they are measured, so dispersion phenomena result. Δn is greater for crystalline arrangements of the lipid chains than for more fluid, disordered ones where the chain orientation is less well defined. The disk membrane is not only composed of lipid, however: about half the volume is protein, which may also be anisotropically arranged. The birefringence resulting from molecular organisation is called intrinsic birefringence, and that arising from the stacked arrangement of rod outer segment membranes is known as form birefringence. The Δn of rods, observed to be positive, is the sum of the form birefringence, negative for a pile of parallel sheets, and the intrinsic component, which must therefore be positive and larger in magnitude than the form component (Schmidt, *Kolloid-zeitschrift*, **85**, 137; 1938). It is the intrinsic Δn which tells us about the molecular orientations in disk membrane, although it cannot distinguish between the membrane constituents. Form and intrinsic contributions to Δn can in

principle be separated, if $n_{\text{cytoplasm}}$ can be varied independently of n_{membrane} , until the form component is eliminated. This requires that the methods used to vary $n_{\text{cytoplasm}}$ have no effect on the membrane spacing, structure or thickness.

Liebman *et al.* first recorded a birefringence spectrum of dark adapted rods in the visible region, then followed the changes in Δn during bleaching. They found a birefringence change appearing at about the rate of the metarhodopsin I $\rightarrow$ II step, the 'birefringence transient' (BRT). The figure is a schematic diagram of the fast birefringence change plotted against wavelength. This curve can be fitted by the sum of two components, one resulting from the optical effects of bleaching, and the other directly caused by changes in the membrane. The first component can be calculated theoretically, and it has the shape of the

curve shown, but its position is shifted upwards so that it is centred on the axis at zero BRT. The observed change is indicated by the heavier portion of the curve. Once the effect of pigment absorption loss is accounted for, what is left is simply a constant (over the wavelength band measured) birefringence loss. This birefringence loss is attributed to a change in the intrinsic birefringence of the membrane. As in the case of nerve, there are many ways of explaining this change in terms of molecular events. It could be due to any combination of small reorientations of lipid or protein, or slightly increased disorder. Nevertheless, Liebman *et al.* have detected a small change which may signal the start of the visual impulse.

HELEN SAIBIL

Astrophysics and energy from black holes

EVER since Einstein's discovery of the famous equation $E=mc^2$ the direct conversion of mass into energy has remained man's most hopeful source of power. Atomic fission reactors, and a possible future generation of fusion devices, manage to achieve the remarkably good efficiency (by terrestrial standards) of converting about 1% of nuclear rest mass into energy. But there almost certainly lurks in the Universe a source of mass conversion with a theoretical efficiency of up to 100%. This source is the famous spinning black hole.

It is now generally appreciated that black holes may only be characterised by three parameters; mass, electric charge and angular momentum. Other than this all black holes appear to the outside world to be identical, whatever their internal constitution. Black holes with rotation possess a number of strange properties which are not shared by their nonrotating counterparts. For example, suppose an inquisitive explorer were to take a trip in a rocket ship near the surface of a rotating black hole. As he approached closer, he would observe that the effect of the enormously compact mass was to try to drag his rocket around with it as it rotated. More and more fuel would need to be expended to resist this effect until, at a certain distance from the object, no amount of rocket power in the universe could prevent the ship from being dragged around in circles. The explorer has entered a new region, the ergosphere, where it is impossible to be 'at rest'. Inside the ergosphere is an event horizon, which if crossed will forbid an object to leave again. Anything which arrives inside this horizon cannot by any power available prevent itself from falling towards the centre of the black hole. But an object which has only ventured as far as the ergosphere can not only escape to the outside world again but actually do so with the bonus of more energy than it went in with. Hence the name 'ergosphere'.

The precise details of this energy-generating process were discovered by Penrose in 1969 and are based on the fact that a particle in the ergosphere is able to move along a trajectory which has negative total energy relative to a distant observer. Consequently, if a particle falls into the ergosphere and breaks into two pieces such that one of these pieces moves on a negative energy trajectory, then the other piece will re-emerge with an energy which is greater than the original combined energies of both pieces, while the sacrificed piece will plunge on into the hole, the negative energy it carries thereby reducing the total mass and angular momentum of the black hole. The process cannot, how-

ever, be repeated indefinitely so that the black hole disappears altogether. Eventually, when the angular momentum has been reduced to zero, no further energy may be extracted in this way.

A rather fanciful practical application of the Penrose process has been discussed by Misner, Thorne and Wheeler (*Gravitation*, 908; Freeman, San Francisco, 1973). An advanced civilisation could build a city around a rotating black hole, and daily dispose of its waste by dropping it in special vessels into the ergosphere, where the waste would be tipped out along a negative energy trajectory, thereby not only obligingly disposing of itself in the most effective dustbin in the Universe, but in addition supplying the re-emerging vessel with an energy equivalent of the entire waste rest mass, plus some of the rest mass of the black hole as well.

As well as contributing to the solution of cosmic ecology problems, the Penrose process might serve as a mechanism for producing high velocity jets in various astrophysical situations. A black hole at the centre of a galaxy could act as a repository for nearby stars, which might fragment appropriately on their way through the ergosphere, thereby imparting a very high velocity to one of the fragments, which would then emerge in the form of a jet.

Strict limits on the energetics of jets formed in this way have been deduced by Wald, of the University of Maryland, in the *Astrophysical Journal* (191, 231; 1974). The argument used involves a simple comparison of the breakup process in ordinary special relativity with that occurring in the ergosphere of a rotating black hole.

The results of this comparison are disappointing for the astrophysicist. So similar are the limiting expressions in the two cases that the energy of the emitted fragment which is attainable is not greatly different whether the black hole is there or not. For example, an object simply falling from rest a great distance away could initiate the ejection of a fragment with a velocity at most $1+\nu\sqrt{2}$ compared with the special relativistic value of ν . The emitted fragment can never be relativistic unless the incident body or the breakup velocity are themselves relativistic.

Thus, although rotating black holes may well be responsible for triggering violent explosions at the centres of galaxies, it seems that the Penrose energy is unlikely to be making a major contribution to such events after all.

P. C. W. DAVIES

Hunt the helix

from E. G. Richards

No sooner was the first primary structure of transfer RNA worked out, than the clover leaf model for its secondary structure was proposed. This has become an article of faith for molecular biologists and all of the 60 sequences now available conform to it. When the sequence of 5S RNA from *Escherichia coli* was published in 1967, several authors leapt to the challenge and published models for its secondary structure although computer investigations suggested that there were several million possibilities. The favourite tack today is to devise experiments that eliminate at least some of these. Kearns and Wong (*J. molec. Biol.*, **87**, 755-774; 1974) have attempted to go one better and deduce a unique structure from the application of the nuclear magnetic resonance (NMR) method which they and others have so successfully applied to tRNA structure.

Resonances in the range 12-15 p.p.m. arise from hydrogen bonded ring N-H protons from U or G so that the number of resonances gives the number of AU and GC pairs—at least it does if GU pairs are ignored since this pair has two H-bonded ring N-H protons, but this possibility is glossed over. Kearns and Wong find that at 35°C there are 28 ± 3 base pairs on this basis of which but 4 are AU. As the temperature is increased about 10 resonances have melted out at 52°C to leave a 'core' structure which is stable to 62°C. By 73°C a further 9 or so have gone to leave about 9 base pairs. This maximal figure of 28 pairs is at variance with estimations obtained from the analysis of various optical properties which are in the range 38-44 pairs. These optical methods are fraught with assumptions that are difficult to test adequately and it is reasonable to accept Kearns and Wong's result as being the more reliable.

Kearns and Wong have attempted to delineate the precise base pairing scheme involved. To do this they have employed explicit semi-empirical rules for calculating the precise shift expected of an AU or a GC base pair in a variety of different helical environments defined by its nearest neighbours. They thus show that the structure left at 73°C gives a spectrum in close agreement with that expected of the longest and presumably most stable helical region that it is possible to form from the sequence. They have gone on to list all possible helical regions in order of length and GC content and worked out the position of the resonances of each. They thus show that the 'core' present at 52°C could contain the two most stable helices, H₁

and H₂. If these two are present, H₃-H₁₀ are automatically excluded since they each involve residues in common with the first two; moreover, H₆-H₁₀ give resonances that are inconsistent with the 52°C spectrum. The next most stable contender is H₁₁ and the spectrum comprised of the H₁, H₂ and H₁₁ contributions is in nice agreement with the 52°C result and they conclude that the core contains these regions. This core structure is of some interest in that it is essentially three dimensional unlike most proposals for RNA secondary structure which can be neatly drawn on a flat page of a journal without the chain crossing itself—a restriction that would seem to be purely aesthetic in origin.

The helices comprising the core rule out all but 12 of the remaining possible helices and calculation of the spectra of these eliminates all but 6 from further consideration. Not all combinations of these 6 are possible but the NMR data do not allow further progress. Kearns and Wong thus invoke the results of oligonucleotide binding experiments that imply that certain regions of the sequence are non-helical. This serves to eliminate all but H₁₇ and H₇₉. They thus provisionally conclude that the full low temperature structure contains the five helical regions H₁, H₂, H₁₁, H₁₇, and H₇₉ giving a total of 28 base pairs of which 11 are AU. The discrepancy in the number of AU pairs is perhaps not too serious in that the partition of the low temperature spectrum into AU and GC regions is subject to error. The question may be raised as to whether this structure corresponds to the native or denatured forms of the molecule; Kearns and Wong claim that their results are independent of the nature of the solvent and the mode of preparation suggests that at least some of their solutions contained a mixture of both forms. It may be that the two forms are not distinguished by their technique even though it is almost certain that they have different base pairing schemes.

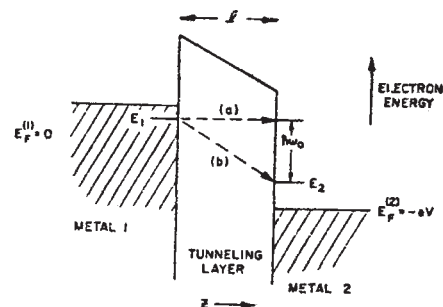
A further question is whether their final model is compatible with other available experimental data which they do not invoke. The results of limited nuclease attack have been interpreted to imply that certain pairs of regions of the structure are not hydrogen bonded to each other but, alas, it can be readily shown that Kearns and Wong's model is incompatible with these interpretations. Maybe these assumptions are at fault or maybe Kearns and Wong were too simplistic in ignoring GU pairs, looped out bases and other possible structural complications. Enthusiasts for these sorts of games await further developments with eager anticipation.

Solid state test tubes

from our Solid State Physics Correspondent

MANY solid state scientists have wondered whether the particular physical system with which they work (possibly a semiconductor surface or a glassy matrix) might be so sensitive to the activity of added chemicals that they could identify the chemical precisely by means of a simple electrical measurement on that solid. The result would be a novel way of following reactions occurring in or on a solid—a sort of solid state test tube.

One example of the fulfilment of such a wish has been developing over the last few years. Giaever discovered in 1960 that the I-V characteristic of metal-insulator-metal (MIM) sandwiches was controlled by tunnelling if the insulator was only 20 Å thick. He realised that the non-ohmic behaviour of the I-V characteristic happened because of the transitions of the tunnelling electron from an energy level in one metal to a different level in the other and that energy-band information—for example the size of a superconducting energy gaps—could be extracted from these I-V characteristics. In fact, the I-V curve not only contained band information but also showed fine structure arising from phonon transitions and other vibrational processes (Jaklevic and Lambe, *Phys. Rev. Lett.* **17**, 1139; 1966). In



Pb-Al₂O₃-Al sandwiches an Al phonon transition can be identified and, if water is present, then the effect of OH bond vibrations are seen. The reason is that the tunnelling electron, driven by the applied field, can either make a loss-free transition to a state of the same energy on the opposite side of the barrier (E_1 in the figure) or it can make a transition to a lower level, E_2 , provided it can lose exactly the right amount of energy ($\hbar\omega_0$ in the figure). This energy can be transmitted to the O-H group by inelastic transfer and the transition provides an increase in the 'inelastic tunnelling current' at a potential corresponding to $\hbar\omega_0$.

It must then have occurred to Lambe

and Jaklevic: "If it works with the water impurity, why not with other impurities doped into the insulator lattice or even simply spotted into the surface?" Surprisingly, organic impurities did indeed couple well with the tunnelling electrons and measurements of the fine structure of the I-V curve, with very sensitive amplification and differentiation, yielded a spectrum of the bending, rocking and stretching bands of organic groups which was a near perfect analogue of the infrared or Raman spectrum with only a few special solid state effects to be accounted for. Lambe and Jaklevic demonstrated this for simple volatile organic liquids such as acetone and showed that one monolayer of molecules gave suitably strong peaks but that, for sharpness, the sample must be cooled well below the temperature of liquid nitrogen (*Phys. Rev.*, **165**, 821; 1968). The peaks seen in the plot of d^2I/dV^2 , where I is the tunnelling current for an applied voltage V , correspond very closely to the infrared absorption peaks if the voltage scale is regarded as an energy scale in eV. For example, the peak of the O-H bending band appears in both measurements at precisely 0.118 eV.

Simonsen and Coleman, of the University of Virginia (*Nature*, **244**, 219; 1973) then developed a routine system for depositing thin layers of many organic solids by evaporation, using a special apparatus which allowed all MIM device fabrication and sample deposition to be done during one vacuum pump-down. At this point, it was certainly true to say that a very original, special and useful method of vibrational spectroscopy had been added to the infrared and Raman methods. A crude 'solid state test tube' had been developed which would yield a vibrational spectrum without costly infrared optics.

More compounds were, of course, applied to this test cell. And it must be said that, as work progressed, the doubts of the sceptics were less and less justified. Within the range 0.03 to 0.55 eV (300 to 4,400 cm^{-1}) all the correct bands turn up. Coleman and Hansma (*Science*, **184**, 1369; 1974) have now extended the work to some large organic compounds which can only be applied from solution. The success of this 'solution doping' is a breakthrough in that very many more compounds can now be studied and, as a speculation, perhaps reacting solutions can be applied to the film and reaction products identified by the vibrational spectra. Since only a monolayer is required and the MIM devices are 1 mm square, the arrangement can be operated with sub-microgram quantities of material. Of course, the compounds must survive the process of drying on to an

anodic Al_2O_3 film and the subsequent evaporation of a thin overlying metal film. Even large molecules such as calf thymus DNA and *E. coli* RNA seem, however, to survive this treatment since the tunnelling spectra exhibit the expected phosphate vibrations, ring distortions and other purine, pyrimidine and sugar group vibrations. There must, in some cases, be surface reactions and, at the very least, frequency shifts because of absorptive forces and the effects of cooling. These effects will then limit the delicacy with which the method can be used as an absolute sensor of bond vibrations and may also exclude the examination of easily altered compounds such as polypeptides (there is ominous silence on this class of compound).

Thus the inelastic electron tunnelling method, although still requiring much development, is probably the first of a whole range of techniques in which the behaviour of carriers in solids is used to recognise chemical characteristics of juxtaposed molecules by quantum mechanical coupling.

Competition in the laboratory

from a Correspondent

It is common knowledge that an ecologist's view of nature is coloured strongly by the particular species he or she works with. Mammologists emphasise the role of aggression; ornithologists the importance of dispersal ability; and entomologists the extinction of local populations. One example of this phenomenon appears in discussions of competition between species. Some ecologists have argued the coexistence of two species on a single limiting resource is impossible (Gause's Exclusion Principle) and have supported this view with laboratory experiments (Gause, *The Struggle for Existence*, Dover, New York; 1958).

Others have rejected Gause's Principle as being unnecessary and have supported their claim also with laboratory experiments (Ayala, *Nature*, **224**, 1076; 1969). Wallace (*Ecology*, **55**, 245; 1974), in the most extensive series to date of experiments on animal competition in the laboratory, has shown how difficult it is to subscribe to any single view of competition, even when considering an apparently similar group of species in the simplest possible conditions.

Wallace has examined in detail the competition within and between eight species of *Drosophila* maintained on laboratory medium. Although there is no reason to assume that the species are very different in their ecological requirements, Wallace found several different types of interactions. Some pairs interfered with each other, as

would be expected; but others appeared not to interact and others even to facilitate each other, permitting each to maintain higher numbers than it could alone. When there was interference, it sometimes affected both species and sometimes only one of the pair.

Wallace's experiments make it difficult either to accept or reject Gause's Principle. Although it is probably true that two species cannot coexist on a single resource, Wallace's results clearly indicate that, in advance, it is impossible to decide what constitutes a single resource. To describe the competition between several pairs of species, an individual of one species could not be replaced by a fractional equivalent of the other species. That undermines the basis for much of the theory which supports Gause's Principle (MacArthur, *Geographical Ecology*, Harper and Row, New York; 1972). Certainly the construction and application of predictive models of competition between species which are based on the existence of a single limiting resource will prove to be very difficult unless competition in nature is considerably simpler than in Wallace's laboratory.

Self protection in neuromuscular transmission

from our Insect Physiology Correspondent

DURING recent years there has been accumulating evidence that L-glutamate serves as the neuromuscular transmitter substance of arthropods, taking the place of acetylcholine in vertebrates in this respect. The evidence has not yet convinced all authors: some still maintain that glutamate is merely a non-specific agent which will depolarise muscle fibres and ultimately desensitise the receptor sites. One of the unsolved problems lies in the failure of the muscles in arthropods to be affected by the L-glutamate normally present in the blood. How this is prevented has remained something of a mystery.

Murdock and Chapman (*J. exp. Biol.*, **60**, 783; 1974) have made quantitative measurements of the concentration of L-glutamate in the blood plasma of the crayfish *Astacus*, the tarantula *Dugesia* and the locust *Locusta*. In the crustacean and in the spider this concentration was found to be so low that even tenfold the normal level, applied in saline, had no effect on neuromuscular transmission, although higher concentrations did cause depression. On the other hand the average concentration of L-glutamate in *Locusta* is sufficient to depress

transmission; and slightly increased concentrations, which may occur naturally, will cause marked depolarisation. The authors discuss various possible explanations of the indifference of the locust to its circulating glutamate.

In the same issue of the journal (*ibid.*, 673) Clements and May investigate this problem in great detail in the locust *Schistocerca*. They could obtain no evidence of sequestration of glutamate by haemocytes, or of binding of glutamate by haemolymph proteins. On the other hand divalent ions, calcium and magnesium, in the haemolymph do certainly form stable complexes with amino acids and the authors estimate that the level of free glutamate is probably reduced about 25% by binding to the divalent metal ions. Both increasing the calcium concentration in the test saline, and introducing magnesium ions, made nerve-muscle preparations less sensitive to glutamate. Furthermore, these authors consistently found that isolated preparations of the retractor muscle of the claw were much more sensitive to glutamate when dissected out than when perfused in the uninjured femur. The dissected preparations studied in sections showed that the connective tissue sheath of the muscle fibres was disrupted, and ready access given to the spaces between the fibres where the neuromuscular junctions lie. They conclude that the connective tissue sheath serves as protective barrier against glutamate in the haemolymph.

Clements and May are inclined to accept the view that glutamate is normally responsible in insects for the transient depolarisation of the post-synaptic membrane that follows nervous stimulation. But, as Murdock and Chapman point out, even if it does not have this physiological role, the neuromuscular junctions must be protected from the action of unwanted glutamate.

Oscillating yield in a plastic

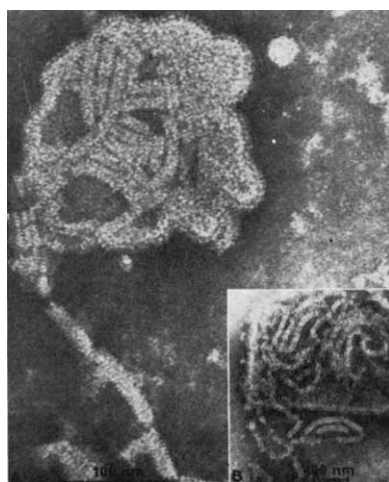
from Robert W. Cahn

SOME years ago, a group of Russian polymer scientists (Andrianova *et al.*, *J. Polym. Sci.*, A2, 9, 1919; 1971) discovered a novel form of behaviour in several polymers. The neck which forms during tensile straining of the amorphous polymer turned out to be made up of successive bands of translucent and opaque material, and the stress-strain curve showed corresponding strain oscillations. The opaque material had evidently crystallised.

This phenomenon of 'oscillating necking' has been theoretically analysed by

Barenblatt in the Soviet Union (*Mekhanika Tverdogo Tela*, No. 5, 121; 1970) and to back up this analysis, Roseen of AB Atomenergi in Sweden has adopted an unusual experimental approach (*J. Mater. Sci.*, 9, 929; 1974). He examined a tensile specimen of amorphous PETP during tensile deformation by means of an infrared Thermovision camera, which records instantaneous temperature distributions. Strips of high temperature (up to 95° C) develop in association with the opaque crystalline strips. The calibration and the resolving power of this camera are set out in some detail. It seems, therefore, that during oscillating necking, a narrow layer of crystalline material forms internally (micrographically it was shown that the surface remains amorphous). Crystallisation seems to be the result of rapid local deformation, which adiabatically heats a narrow front of polymer. The crystalline material is much less ductile than the amorphous matrix; plastic flow and its associated heat generation slows down the stress rises and the cycle repeats.

Thus, polymers may show behaviour superficially similar to the discontinuous yielding long familiar in certain alloys.



Viruses naked and clothed

Two views of paramyxoviruses. A, Newcastle disease virus, a pathogen of chickens. The protein envelope has been broken to allow the escape of part of the nucleocapsid. B, Nucleocapsid from Sendai virus, the favourite of those who fuse cells. The nucleocapsid in both viruses consists of one long single-stranded RNA molecule around which identical protein subunits are arranged. The periodicity of the helix thus formed is visible in B. Photograph from *Virus Structure* by Robert W. Horne (Academic Press, New York and London, 1974).

This is known as the Portevin-Le Chatelier effect and is most familiar in mild steels. In essence, it is caused by the cyclic locking of dislocations by readily mobile impurity atoms (normally carbon or nitrogen). A group of dislocations breaks free, the impurity atoms catch up and lock them afresh, and the cycle repeats.

Roseen has confirmed the postulated difference in tensile behaviour between amorphous and crystalline polymer by examining the effect of intentional heat treatment of the polymer on the form of the tensile curve. Here is another unfamiliar point of similarity between metals and polymers: it is second nature to a metallurgist to examine the effect of systematic heat treatments on the mechanical behaviour of an alloy, but this is not yet a normal experiment for a polymer scientist.

One lesson from this intriguing piece of work is that it can be illuminating for a polymer scientist to know something of physical metallurgy. But it would be hubris to leave it at that. Increasingly, metallurgists have something to learn from polymer scientists, as concerns both phenomena and experimental techniques. It is time that 'polymer crystallisation' became a normal part of the syllabus of students of metallurgy and materials science.

Frenkel pairs in zinc selenide

from John Walker

To account for the electrical conductivity observed in some ionic solids, Frenkel suggested in 1926 that a small percentage of ions could leave their lattice sites and migrate through the crystal. The point defects so formed, the lattice vacancies and their corresponding interstitial partners, have hence been called Frenkel defects. The concept has been widely used during the past 48 years, but until recently no Frenkel defect had been observed directly. Watkins (*Phys. Rev. Lett.*, 33, 223; 1974) has now remedied the deficiency.

After irradiating zinc selenide with 1.5 Mev electrons at a temperature of 20 K, Watkins observed a family of similar spin resonance spectra which he labelled V, V^I, V^{II}, V^{III}, V^{IV}. The V spectrum had previously been assigned to a hole localised on one of the four selenium neighbours to a zinc vacancy. By mechanically compressing the crystal Watkins found that he could make the hole jump freely between the four selenium atoms. (In the language of the trade, a [111] uniaxial stress makes that particular Jahn-Teller distortion energetically unfavourable and the defect flips over into one of the other <111> orientations.)

The other spectra are so similar to V that Watkins also assigns them to zinc vacancies. But the V^I centres do not reorient under uniaxial stress. They are therefore locked in position in the strain field of a nearby defect—the zinc atom which has been ejected to create the vacancy. It occupies the interstitial site nearest to the vacancy.

Warming the crystal above 20 K causes the interstitial zinc atom to move to a new site, behind one of the four selenium atoms surrounding the vacancy. This configuration, called V^{II} , is of lower energy than V^I because the polarisable selenium atom is between the vacancy and the interstitial. In V^{III} the interstitial lies in the $\langle 100 \rangle$ direction. No model was proposed for V^{IV} , the spectrum of which is very weak.

Directional effects observed during irradiation confirm the models. For example, when the electron beam direction is $[111]$, more V^I defects are observed in the $[111]$ configuration than in other $\langle 111 \rangle$ orientations.

Another interesting observation is that the isolated vacancy V is not produced linearly with electron dose. The creation rate follows a square (or perhaps a cube) law. This suggests a two-stage process, the formation of close-pair Frenkel defects being the first step.

The direct observation of vacancy-interstitial pairs in a solid is an interesting confirmation of Frenkel's original suggestion, although its impact is perhaps lessened by our present awareness of the richness and complexity of point defects created by irradiation. (An awareness due in great measure to Watkins, one might add.)

The lessons for *aficionados* of spin resonance are twofold; look for these Frenkel defects in other materials; and use the uniaxial stress technique, without which their identification in zinc selenide would have been much more difficult.

Components of the amoeba membrane

from a Correspondent

FASCINATING details of the surface structure of some primitive animal cells, amoebae and sea anemones, have recently come to light. They provide evidence for new classes of polyanions that may serve functions in primitive organisms, similar to those performed by glycoproteins containing sialic acid and the gangliosides and proteoglycans of metazoal cell surfaces.

Korn and his colleagues have been studying *Acanthamoeba castellanii*, a small free living soil amoeba that

grows well on a soluble medium. About a third of the membrane is made up of a unique component that is extracted into solution with 8 M urea, or better, aqueous butanol, and is easily purified free of a non-glycosylated protein fraction. The new component contains neutral sugars, mainly mannose glucose and xylose, about 14% of fatty acids that include unusual branched chain 2-hydroxy fatty acids, and aminophosphonic acids. Of these last derivatives, the parent compound but not 1-hydroxyl-2-aminoethyl phosphonic acid are known constituents of lipids in various species of lower marine invertebrates but perversely not to any significant extent in amoebae.

Clearly the lipophosphoglycan described by Korn and his colleagues Dearborn and Wright (*J. biol. Chem.*, **249**, 3335-3341; 1974; **249**, 3342-3346; 1974) is an integral component of the membrane and presumably is anchored into it by association of the fatty acids with the phospholipid-sterol matrix of the membrane. Brief mention is made of unpublished electron microscopic evidence by Bowers and Korn that the polar moieties of lipophosphoglycan including phosphate groups and concanavalin A reactive sites (α -mannosyl groups) are exposed on both sides of the amoeba surface membrane.

The exact structure of this polymer is unknown. If the sugars are in normal glycosidic linkage, a core structure of galactosamine residues and aminophosphonic acids, both perhaps substituted with fatty acids and forming the attachment sites for the neutral sugars, seems to be most likely. The linkages between the constituents of the 'core' region are not known except phosphoamide bonds are excluded since these would be labile to acid hydrolysis and this is not observed.

In another publication Allen *et al.* (*J. Cell Biol.*, **60**, 26-38; 1974) describe somewhat similar material at the surface of a uninucleate, freshwater phagocytic amoeba *T1D13*. The only anionic group detected by Allen *et al.* at the cell surface is phosphate which accounts for more than 10% of the isolated surface membrane. Most of this phosphate (90-95%) has been isolated as a polymer in association with *neo*-inositol. Amino phosphonic acids were not detected. The large sugar content, mainly mannose rhamnose and glucose, of these plasma membranes was isolated separately in an uncharacterised fraction that seems to contain appreciable organic phosphate. It seems that these two fractions may be more closely related in the native structure, and perhaps are hydrolytic fragments produced during isolation (a part of the phosphate is very acid labile according to Allen *et al.* whereas the remaining phosphate

is much more stable; the pronounced stability to acid hydrolysis of C-P bands as in phosphonic acids is a characteristic of the Korn polymer). Some structural similarity between the undegraded phosphorylated polymers of *Amoeba T1D13* and *Acanthamoeba* cannot therefore be totally excluded particularly since the analysis of Korn and his associates for lipophosphoglycan purified from *Acanthamoeba* still leaves substantial leeway for inclusion of a polyol such as *neo*-inositol.

The clearest existing analogy to the amoeba lipophosphoglycan is perhaps the lipopolysaccharide of certain Gram negative bacteria. Both sorts of compounds contain fatty acids including hydroxy fatty acids, and neutral and aminosugars. The bacterial polymer contains ethanolaminephosphate, the ester analogue of 2-aminoethyl phosphonate. The fraction isolated by Allen *et al.* perhaps suggests superficially a resemblance to another ubiquitous class of bacterial polymers, the teichoic acids which are based on ribitol or glycerol phosphate derivatives. Of course, it may turn out that the amoebae polymers are unique structures with no known biological precedents.

Aminophosphonic acids also turn up in phosphoglycoproteins isolated from *Metridium dionthus* (Hilderbrand *et al.*, *Biochemistry*, **12**, 4756-4762; 1973). These partially purified fractions contain 2-aminoethyl phosphonic acid, neutral sugars and hexosamines but apparently not significant amounts of fatty acids. The structural relationship of these polymers to the lipophosphoglycan and in particular the method of attachment of the chains containing organic phosphorus to the polypeptide moiety will be of interest.

The prevalence of surface negatively-charged polymers of diverse structure in the lower invertebrates suggests a positive function in the ecological success of these organisms. This role may be as a mechanism providing a stable environment surrounding the cell, and may be related to the ability of the amoebae surface to absorb large quantities of various cations. This function in turn has relevance to subsequent pinocytotic events during feeding and in the uptake of nutrients under culture conditions. The existence of an anionic surface that could function as a buffering system could also form a valuable protective role against foreign cationic substances present in the surrounding medium (sea water). Alternatively, if present in the buccal cavity the phosphonate compounds may protect the organism from self dissolution by degradative enzymes spilled over from the digestive tract.

articles

Jupiter after Pioneer: a progress report

Thomas R. McDonough

School of Electrical Engineering, Cornell University, Ithaca, New York 14850

"We now come to a much more magnificent planet, Jupiter, the largest of them all . . ." So wrote Sir John Herschel in A Treatise on Astronomy¹. Now, after a decade in which space programmes have concentrated on the inner planets, a spacecraft has been sent to Jupiter. This article describes how the data sent back are changing our ideas about the planet.

In December 1973, Pioneer 10 became the first artefact of man to reach the environs of Jupiter. In the two weeks that it spent travelling through the Jovian magnetosphere, it sent back more than 300 pictures of Jupiter, as well as measurements of magnetic fields, energetic particles, plasma, infrared and ultraviolet emissions, and micrometeoroids. The spacecraft passed within $1.8R_J$ of the cloudtops of Jupiter (R_J , the radius of Jupiter, is 71,600 km). Preliminary reports have now been published²⁻¹⁸, and here I present a progress report summarising them, describing some of the first theoretical interpretations, and suggesting some of the gaps that remain in our understanding of Jupiter.

Radio occultations

The temperature structure of the Jovian atmosphere over more than 100 km of altitude has been inferred by Kliore *et al.*¹⁶ from a study of the Pioneer S-band (2,200 Mhz) signals as the spacecraft was occulted by Jupiter (see Fig. 1). They found the temperature to behave quite differently from all published models, with a very strong temperature inversion at about 20 mbar pressure and 280 K temperature, which may be explained by absorption of solar radiation by dust^{16,19}. They also expect to derive an electron-density profile of the Jovian ionosphere, but it is complicated by multipath propagation¹⁶. This will be a welcome addition to the many ionosphere models that have lacked any data by which to be tested.

Pioneer's trajectory was designed so that it would be occulted by the Jovian satellite Io. It had been suspected for several years that Io might have an atmosphere, because Io is sometimes brighter after passing behind Jupiter as if snow had fallen during the passage through Jupiter's shadow²⁰⁻²². Recently, evidence of atomic sodium gas at Io has been found by R. A. Brown^{18,23}. Analysing the Io occultation, the Kliore group discovered that Io has an ionosphere, with a peak density of $6 \times 10^4 \text{ cm}^{-3}$ at an altitude of $\sim 100 \text{ km}$ (see Fig. 2). They estimated theoretically that this could imply a neutral atmosphere with a density at the

Io surface of 10^{-8} to 10^{-10} bar. They propose that Io has a new kind of ionosphere, reaching from the surface up to 700 km.

Ultraviolet photometer

A year ago it was predicted that if any of the Galilean satellites have atmospheres, gases escaping from them could form large toroidal rings around the planet^{24,25}. Judge and Carlson¹¹ using Pioneer's ultraviolet photometer, found Lyman- α emissions (attributed to atomic hydrogen) of several hundred rayleighs intensity from the equatorial plane of Jupiter at a time when neither Jupiter nor its satellites were in the field of view, and they tentatively interpret the radiation as originating in such a toroid. They also made the surprising discovery that Io seems to have a Lyman- α brightness of 10^4 rayleighs, corresponding to a column excitation rate of $10^{10} \text{ photons cm}^{-2} \text{ s}^{-1}$, an order of magnitude brighter than Jupiter at this wavelength. Although such Lyman- α radiation is usually the result of scattering of solar ultraviolet by atomic hydrogen, they

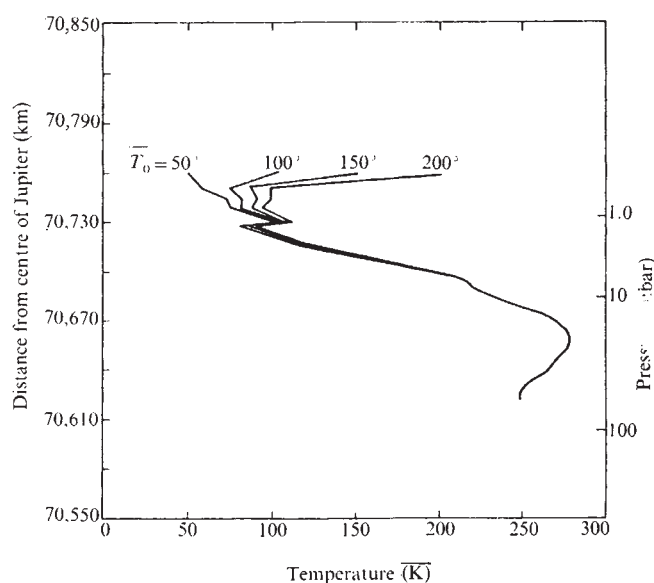


Fig. 1 The temperature profile of Jupiter's atmosphere as deduced by Kliore *et al.*¹⁶ from the Pioneer 10 radio occultation. A composition of 15% He and 85% H_2 by volume was assumed, and the computation was performed for four different initial temperatures, T_0 .

suggest that this extraordinary brightness may be due to auroras on Io.

The presence of hydrogen on Jupiter has long been known, but helium had never been detected until Pioneer's arrival. Judge and Carlson's photometer detected ~ 15 rayleighs of the He 584 Å emission line. Their Lyman- α (1216 Å) channel measured a Jovian brightness of about 10^3 rayleighs, attributed to the glow of atomic hydrogen, a lower value than sounding-rocket measurements of Rottman *et al.*²⁶. Derivation of the He/H ratio from the ultraviolet measurements awaits detailed model-atmosphere calculations, but the 1971 model of Carlson and Judge²⁷ suggests, for the observed helium emission, a value of He/H₂ $\sim 50\%$ in the lower atmosphere.

Infrared radiometer

Chase *et al.*¹⁰ (G. Münch, principal investigator) mapped Jupiter at wavelengths of 20 and 40 μm , with the Pioneer infrared radiometer. The 20 μm channel contains the rotational component of the H₂ spectrum, and the 40 μm one the translational component. The latter is dependent on the partial pressure of He, so for the first time it is possible to measure the He/H₂ ratio on Jupiter, although the ratio is somewhat model-dependent. Theoretical estimates of the abundance of Jovian helium have ranged from the cosmic abundance, in which He/H $\sim 10\%$ by number, up to a model in which helium is the major constituent and hydrogen a minor one²⁸. This abundance is an important input to models of the Jovian interior and atmosphere. The Chase group calculates He/H₂ ratios (by number) from 0.6 to 0.8, that is, He/H is from 0.3 to 0.4, somewhat higher than most theoreticians expected, and higher than the values emerging from stellar occultation studies (L. Wasserman, private communication).

Measured infrared brightness temperatures ranged from 115 K to 145 K, and seem to be roughly correlated with the visible belts and zones. For the first time ever, the rear of Jupiter was observed, and, as expected, the planet was found to radiate as much infrared from its dark side as from the front. Chase *et al.* compute that Jupiter radiates about twice as much energy as it receives from the Sun, a result somewhat smaller than the aircraft measurements of Aumann *et al.*²⁹ but removing at last any uncertainty about the far-side emission. Jupiter radiates like a feeble star.

Imaging photopolarimeter and television

The imaging photopolarimeter of Gehrels *et al.*¹² contained a 2.5 cm telescope which made maps of Jupiter in blue and red light, polarised and depolarised, as the spacecraft spun. Some measurements were also made of the Galilean satellites. The most striking early results were the hundreds of photographs that resulted from the spin-scans of the planet, giving higher resolution pictures of the planet than had previously been possible. Four "small" (4,000 km) bright spots have been observed, as have several bright swirls. Two of the light-coloured zones of Jupiter show a distinct wave pattern. The border of the Great Red Spot is dark, and sharp to the limit of resolution.

Gehrels *et al.* found that the blue polarisation varied from several per cent. at the centre of the disk to $\sim 40\%$ at the poles. These high polarisations are believed to be due to Rayleigh scattering, and Gehrels *et al.* expect to use them to measure the amount of gas above the clouds. It

may be possible to derive the size, shape, and refractive index of the cloud particles now that we have measurements over the large phase angles impossible to obtain from Earth.

The magnetometer

The first surprise from Pioneer was the detection of Jupiter's influence on fields and particles well before the expected time. It is known that the solar wind is deflected by the Earth's magnetic field, forming a bow shock, and straightforward scaling implied that the Jovian bow shock should occur at about 50 R_J from the planet³⁰. But the helium vector magnetometer of Smith *et al.*⁵ detected the bow shock at 108 R_J . The magnetopause was observed at 96 R_J , and the magnetic field remained near 5×10^{-5} gauss from there to about 50 R_J with frequent irregular dips to values near 1×10^{-5} gauss.

After 25 R_J , the field began to appear more dipolar.

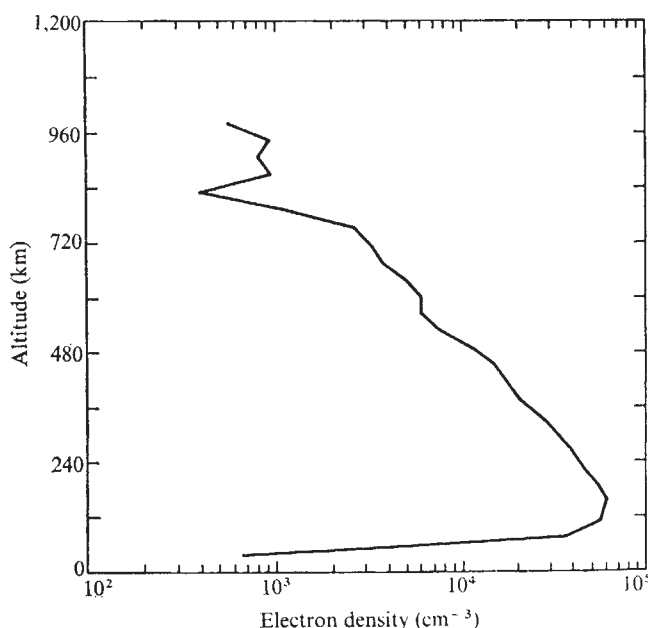


Fig. 2 The ionospheric density of Io deduced from the Pioneer 10 occultation. The solar zenith angle was about 81° and the uncertainty in altitude was approximately ± 40 km.

Using the field values measured when close to the planet, the experimenters estimate the dipole moment of Jupiter to be 4.0 gauss R_J^3 . The dipole is tilted by $\sim 15^\circ$ and displaced by about 0.2 R_J from the centre of the planet, which implies surface fields from ~ 2 to ~ 12 gauss if quadrupole terms are negligible. This is a more tilted and slightly weaker field than had been deduced from terrestrial measurements of the decametric noise bursts, but is not in serious disagreement. The field is upside-down with respect to the Earth's which was also expected.

Smith *et al.* suggest that their observations could be explained by a disk-like magnetosphere, radically different from the Earth's, although they do not rule out the possibility of a thick magnetosphere. The reason for such a non-spherical 'magnetodisk' may be that the density and temperature of the magnetospheric plasma are so great that the magnetic field cannot contain the plasma at distances larger than about 20 R_J . Inside that distance, it would resemble the Earth's magnetosphere, and outside, plasma would be thrown out, forming a magnetodisk something

like the solar wind, with a spiral magnetic field, but which would also have some of the properties of the Earth's magnetotail, such as a neutral sheet.

Plasma analyser and energetic particle detectors

The plasma analyser of Wolfe *et al.*⁴ measured proton spectra from 100 eV to 18 keV, electron spectra from 1 to 500 eV, charged-particle fluxes from 1×10^2 to 3×10^9 cm⁻² s⁻¹, and the plasma flow velocity. Their measurements of the bow shock correlated well with the magnetometer observations. They found that the electron energy tended to peak at about 3 eV within the magnetosphere. The ion energies were too small to register but the authors suggest that the outer magnetosphere of Jupiter is in a high-beta regime—in other words, the thermal energy dominates the magnetic energy.

A most surprising result of the plasma analyser measurements was that the spacecraft apparently passed through the magnetosheath twice while approaching Jupiter, as if there were not one but two magnetospheric bow shocks. The first occurred at about 100 R_J , and the second at about 50 R_J . As the spacecraft left Jupiter three more shocks were observed. Wolfe *et al.* suspect that the solar-wind pressure may have fluctuated, causing the bow-shock distance to vary, but it has also been suggested that this could be an effect of the eccentricity and tilt of the Jovian magnetic dipole. One suspects that if the plasma is ejected into the magnetodisk from near the magnetic equator, the plasma may create giant waves in the magnetodisk, so the spacecraft may have passed through several troughs and crests of a wavy magnetodisk.

The flux of relativistic (≥ 3 MeV) electrons, of protons above 0.5 MeV, and of 1 MeV per nucleon helium nuclei were measured by Simpson *et al.*⁶ with the charged-particle detector, and electrons above 70 keV were observed by Van Allen *et al.*⁷ with the Geiger-tube telescope. The cosmic-ray telescope results reported by Trainor *et al.*⁸ (F. B. McDonald, principal investigator) provided data on high-energy electrons, protons, and helium nuclei, including high-intensity flux measurements of protons from 0.5 to 21 MeV and electrons from 0.1 to 1 MeV. The trapped radiation detector of Fillius and McIlwain⁹ measured fluxes, energy spectra, and angular distributions of 100 keV to 50 MeV electrons and of protons above 70 MeV.

One month before encounter, the Simpson group detected bursts two days long of electrons up to 30 MeV in energy, analogous to precursors of the terrestrial magnetosphere but far more energetic. The flux of relativistic electrons⁶ reached $\sim 5 \times 10^6$ cm⁻² s⁻¹ near closest approach (2.8 R_J), which was about an order of magnitude greater than the upper limit of the Jupiter Radiation Belt Workshop³¹. The Workshop electron model was based to a large extent on terrestrial observations of the radio emissions of Jovian relativistic electrons, and the weaker magnetic field found by Jupiter accounts in part for the underestimate of the electron flux. For protons, there were no such observational data prior to Pioneer, yet the observed⁶ peak flux of $\sim 4 \times 10^6$ protons cm⁻² s⁻¹ was near the Workshop nominal prediction. The proton flux falls off within $\sim 3.5 R_J$, however, which was unexpected.

All four groups found the particles to be highly concentrated near the magnetic equatorial plane from ~ 20 to $\sim 100 R_J$, and remarkably different from the terrestrial magnetosphere. On the outward journey through the magnetodisk, bursts of energetic electrons and protons were found with time scales of minutes, which had great directional anisotropies⁶. The experimenters noted similarities with phenomena of the Earth's magnetotail, which are understandable if the Jovian magnetic field is confined to a disk

outside $\sim 20 R_J$, since one would expect the field to be stretched out in the disk in a manner similar to a magnetotail. They found that the particles are trapped in a manner similar to the Earth's magnetosphere only for distances within about 20 R_J .

Mead and Hess³² had proposed that the Galilean satellites could sweep up some of the energetic particles, and Pioneer fluxes did indeed dip while passing the orbits of Europa, Io, and possibly Ganymede, but not that of the most distant and theoretically least influential, Callisto⁷⁻⁹. Perhaps the proton decrease within 3.5 R_J is related to sweeping-up by the small satellite Amalthea at 2.5 R_J , which was also suggested by Mead and Hess^{6,32}.

Asteroid and meteoroid detectors

Two instruments on Pioneer detected micrometeoroid and larger particles. The photomultiplier telescopes of Soberman *et al.*¹³ detected particles ranging from 35 μ m to 10 cm at distances from 2 to 3.5 AU. The pressure cells of Kinard *et al.*¹⁴ detected particles of mass $\sim 10^{-9}$ g, and showed that the asteroid belt had no more of these particles than the regions before and after the belt. As was expected, the number of particle detections by the latter instrument rose dramatically when it arrived at Jupiter. Kinard *et al.* do not yet know whether the particles are interplanetary ones attracted by Jupiter, or particles actually orbiting the planet.

Trajectory analysis and spacecraft survival

Anderson *et al.*¹⁵ have studied the Doppler shifts of the S-band transmissions of Pioneer 10 to determine the spacecraft trajectory with great precision. They expect to be able to measure the masses of the Galilean satellites to 1% by studying the perturbations they cause on the trajectory. They have already found that Io is about 20% heavier than previously thought, making its density 3.5 g cm⁻³, slightly denser than our own Moon, which places an important constraint on chemical models of its interior. If the pre-Pioneer densities of the other Galilean satellites²¹ are approximately correct, this new Io density implies that the density of Galileans decreases with distance from the planet, analogous to the general behaviour of planet densities with distance from the Sun, which is a clue to the origin and evolution of the Solar System.

Anderson *et al.* also expect to measure several of the higher harmonics of the Jovian gravitational field, giving information on the distribution of mass within Jupiter obtainable in no other way. This should tell us something about whether Jupiter has a core, and what its composition is, and whether there is an internal mass-asymmetry related to the eccentric magnetic field.

An important datum provided by Pioneer 10 was that it survived passage through the asteroid belt and the Jovian magnetosphere. It received an integrated dose of 2×10^3 rads from electrons and 5×10^4 rads from protons greater than 30 MeV, where 500 rads is a lethal human dose¹⁷. This was very nearly the maximum dose that the spacecraft could withstand. In addition to that, it is known that a spacecraft in a Van Allen belt may charge up to potentials large enough to damage the instruments³³, and it has been suggested that this charging phenomenon could be a danger to Pioneer (N. M. Brice, private communication, and unpublished work of F. L. Scarf and R. W. Fredericks). The fact that the spacecraft survived this danger is consistent with the presence of an unexpectedly large density of magnetospheric plasma, whose presence would tend to nullify the charging caused by energetic particles. Fillius

and McIlwain⁹, however, do not rule out the possibility of serious spacecraft charging, which, they point out, may bias charged-particle measurements.

The future

Pioneer 10 has confirmed as inescapable the fact that Jupiter radiates more energy than it receives from the Sun. The source of this radiation is a major theoretical problem. Models of the Jovian interior must not only explain this, but must also explain the eccentricity of the magnetic field and the values of the gravitational parameters of the planet. The observed He/H abundance will serve as a constraint on atmospheric and interior models.

Jovian meteorology is much more complex than had been supposed, and new atmospheric models must be constructed, probably incorporating dust. The multitude of theories of the Great Red Spot may at last be tested against the new data.

It is up to theoreticians to understand why the magnetosphere of Jupiter proved to be so utterly different from what we had expected. At this early date, it seems to be due to unexpectedly large amounts of magnetospheric plasma, so it may be that the rate of plasma input to the magnetosphere is much larger than has been estimated or that the storage time is much greater.

The distribution of energetic electron and proton fluxes can now be tested against existing theories of particle-diffusion mechanisms (refs 34–36 and unpublished work of S. A. Jacques and L. Davis jun.). Interpretation of the decimetric radiation has been complicated by the fact that the synchrotron emission is a function of both the electron energy spectrum and the magnetic field. Now that we know the strength and structure of the magnetic field, we can use the theory of synchrotron radiation to extract the relativistic electron energy spectra from Earth based radio-telescope observations of the decimetric noise. If the deduced energy spectra are consistent with the Pioneer measurements, this will verify the calibration of both the energetic electron sensors and the magnetometer. We can then use past and future earthbound observations of the decimetric noise to measure the relativistic electron energy spectra as a function of time and position in the Jovian Van Allen belts.

Io is now an even more intriguing object than before. It has an atmosphere, an ionosphere, a torus, sodium atoms, brightness fluctuations, possible auroras, and it appears to control the decametric noise bursts. Theories of the interaction of Io with the Jovian magnetic field, designed to explain the radio bursts, must now incorporate a conducting ionosphere. It has even been suggested that Io may be the source of the Van Allen belt electrons¹⁸.

Pioneer 10 is not only the first spacecraft to reach Jupiter, it will be the first to leave the Solar System. It carries on it the famous plaque bearing a drawing of a man and woman, and telling, in the language of mathematical physics, the location of its home planet³⁷.

As Pioneer 10 leaves the Solar System, its near-twin, Pioneer 11, is in the asteroid belt, and is scheduled to arrive at Jupiter next December. At present, NASA has decided to give it a trajectory which would take it to Saturn in 1979, but which might expose it to dangerous radiation levels at Jupiter³⁸. Two Mariner spacecraft are scheduled to be launched in 1977 to visit Jupiter and Saturn.

Many of the discoveries of Pioneer 10 can be economically described by the statement that Jupiter resembles the Sun more than the Earth. In composition, energy production, radio noise, differential rotation, field eccentricity, and the interaction of its plasma with its magnetic field, Jupiter displays truly stellar qualities.

This paper is dedicated to the memory of Professor Neil M. Brice, whose recent tragic death withdrew from the

world a brilliant scientist, a wonderful human being, and a very good friend. Whatever growth as a scientist I may have attained in the last few years, I owe largely to his encouragement, stimulation, enthusiasm, energy and constructive criticism.

I thank the following for useful conversations: Professors P. Gierasch, M. Harwit, and C. Sagan, Drs S. Soter and W. E. Swartz, Messrs B. Gross, R. Reed, and Dr L. Wasserman, of Cornell University; Professor T. Gehrels of the University of Arizona; Dr A. Kliore of the Jet Propulsion Laboratory; Dr J. D. Mihalov of the NASA Ames Research Center; Dr T. D. Parkinson of Kitt Peak National Observatory; and Dr J. A. Simpson of the University of Chicago. This research was sponsored by the NASA Physics and Astronomy Program and the National Science Foundation Atmospheric Sciences Section. I thank Dr Kliore for permission to use the radio-occultation curves of ref. 16.

¹ Herschel, J. F. W., *A Treatise on Astronomy* (London 1833).

² Hall, C. F., *Science*, **183**, 301 (1974).

³ Opp, A. G., *Science*, **183**, 302 (1974).

⁴ Wolfe, J. H., Collard, H. R., Mihalov, J. D., and Intriligator, D. S., *Science*, **183**, 303 (1974).

⁵ Smith, E. J., Davis, L., jun., Jones, D. E., Colburn, D. S., Coleman, P. J., jun., Dyal, P., and Sonett, C. P., *Science*, **183**, 305 (1974).

⁶ Simpson, J. A., Hamilton, D., Lentz, G., McKibben, R. B., Mogro-Campero, A., Perkins, M., Pyle, K. R., Tuzzolino, A. J., and O'Gallagher, J. J., *Science*, **183**, 306 (1974).

⁷ Van Allen, J. A., Baker, D. N., Randall, B. A., Thomsen, M. F., Sentman, D. D., and Flindt, H. R., *Science*, **183**, 309 (1974).

⁸ Trainor, J. H., Teegarden, B. J., Stilwell, D. E., McDonald, F. B., Roelof, E. C., and Webber, W. R., *Science*, **183**, 311 (1974).

⁹ Fillius, R. W., and McIlwain, C. E., *Science*, **183**, 314 (1974).

¹⁰ Chase, S. C., Ruiz, R. D., Münch, G., Neugebauer, G., Schroeder, M., and Trafton, L. M., *Science*, **183**, 315 (1974).

¹¹ Judge, D. L., and Carlson, R. W., *Science*, **183**, 317 (1974).

¹² Gehrels, T., Coffeen, D., Tomasko, M., Dose, L., Swindell, W., Castillo, N., Kendall, J., Clements, A., Hämeen-Anttila, J., Knight, C. K., Blenman, C., Baker, R., Best, G., and Baker, L., *Science*, **183**, 318 (1974).

¹³ Soberman, R. K., Neste, S. L., and Lichtenfeld, K., *Science*, **183**, 320 (1974).

¹⁴ Kinard, W. H., O'Neal, R. L., Alvarez, J. M., and Humes, D. H., *Science*, **183**, 320 (1974).

¹⁵ Anderson, J. D., Null, G. W., and Wong, S. K., *Science*, **183**, 322 (1974).

¹⁶ Kliore, A., Cain, D. L., Fjeldbo, G., Seidel, B. L., and Rasool, S. I., *Science*, **183**, 323 (1974).

¹⁷ Abelson, P. H., *Science*, **183**, 261 (1974).

¹⁸ Metz, W. D., *Science*, **183**, 293 (1974).

¹⁹ Axel, L. A., *Astrophys. J.*, **173**, 451 (1972).

²⁰ Binder, A. B., and Cruikshank, D. P., *Icarus*, **3**, 299 (1964).

²¹ Newburn, R. L., jun., and Gulkis, S., *Space Sci. Rev.*, **13**, 179 (1973).

²² Sinton, W. M., *Icarus*, **20**, 284 (1973).

²³ Brown, R. A., *Proc. IAU Symp. No. 65* (in the press).

²⁴ McDonough, T. R., and Brice, N. M., *Nature*, **242**, 513 (1973).

²⁵ McDonough, T. R., and Brice, N. M., *Icarus*, **20**, 136 (1973).

²⁶ Rottman, G. J., Moos, H. W., and Freer, C. S., *Astrophys. J.*, **184**, L89 (1973).

²⁷ Carlson, R. W., and Judge, D. L., *Planet. space Sci.*, **19**, 327 (1971).

²⁸ Öpik, E. J., *Icarus*, **1**, 200 (1962).

²⁹ Aumann, H. H., Gillespie, C. M., and Low, F. J., *Astrophys. J. Lett.*, **157**, L69 (1969).

³⁰ Carr, T. D., and Gulkis, S., *Ann. Rev. Astr. Astrophys.*, **7**, 577 (1969).

³¹ Beck, A. J., (editor), *Proc. Jupiter Radiation Belt Workshop, Tech. Mem. 33-543* (Jet Propulsion Laboratory, Pasadena, Calif., 1972).

³² Mead, G. D., and Hess, W. N., *J. geophys. Res.*, **78**, 2793 (1973).

³³ De Forest, S. E., *J. geophys. Res.*, **77**, 651 (1972).

³⁴ Brice, N. M., and McDonough, T. R., *Icarus*, **18**, 206 (1973).

³⁵ Brice, N. M., and Ioannidis, G. A., *Icarus*, **13**, 173 (1970).

³⁶ Birmingham, T., Hess, W., Nithrop, T., Baxter, R., and Lojko, M., *J. geophys. Res.*, **79**, 87 (1974).

³⁷ Sagan, C., Sagan, L. S., and Drake, F., *Science*, **175**, 881 (1972).

³⁸ Sullivan, W., *The New York Times*, 59 (February 10, 1974).

Trapped xenon in meteorites

D. D. Sabu,

Department of Chemistry, Grambling College, Grambling, Louisiana 71254

E. W. Hennecke, & O. K. Manuel

Nuclear Division, Department of Chemistry, University of Missouri, Rolla, Missouri 65401

Xenon in meteorites can be resolved into a mixture of component X and trapped xenon with the following composition, ^{124}Xe : ^{126}Xe : ^{128}Xe : ^{130}Xe : ^{131}Xe : ^{132}Xe : ^{134}Xe : ^{136}Xe = 0.0276: 0.0248: 0.501: 1.00: 5.04: 6.19: 2.31: 1.90. This trapped meteoritic xenon is distinct from xenon trapped in the average carbonaceous chondrite which is shown to represent the average composition of the total xenon in meteorites containing various mixtures of component X and trapped meteoritic xenon.

IN 1960, Reynolds¹ reported the discovery of radiogenic ^{129}Xe , the decay product of extinct ^{129}I , in the Richardton chondrite. He also noted a general anomaly pattern across the isotopes of xenon in the Richardton meteorite, and later that year he reported² a similar isotopic anomaly pattern in xenon released from the Murray carbonaceous chondrite. Since Murray contained over 40 times more xenon than Richardton, but less cosmogenic noble gas isotopes than Richardton, Reynolds² concluded that the general anomaly pattern was characteristic of primordial xenon.

Following Reynolds^{1,2} discovery of general isotopic anomalies in meteoritic xenon, there were other attempts to determine accurately the isotopic composition of xenon trapped in meteorites³⁻⁵. Apart from a component similar to atmospheric xenon which is released at low extraction temperatures and is presumably due to contamination of meteorite surfaces by the atmosphere, these studies¹⁻⁵ showed that the xenon trapped in average carbonaceous chondrites (AVCC Xe) may be a mixture of fractionated atmospheric-type xenon plus a fission component. But, the concept of AVCC Xe as primordial, when defined as a mixture of two components, was unclear, and this concept has become increasingly vague as the results of stepwise heating experiments on different carbonaceous chondrites in several laboratories⁶⁻¹³ demonstrate convincingly that the two components of AVCC xenon are inhomogeneously mixed.

Accurate measurements of the isotopic composition of xenon from the Sun might help elucidate the nature of primordial xenon in our Solar System, and a component of solar xenon has recently been identified in the Pesyanoe meteorite¹⁴ and in lunar soil¹⁵⁻²⁰. The general features of this solar xenon can be related to atmospheric xenon by isotopic mass fractionation, although certain xenon isotopes consistently deviate from the general

fractionation pattern¹⁵⁻²². It has been suggested that deviations from the general isotopic fractionation pattern are due to radiogenic ^{129}Xe in the atmosphere^{18,22}, to excess ^{131}Xe and ^{132}Xe in the atmosphere¹⁸, to fissionogenic ^{134}Xe and ^{136}Xe in lunar fines^{16-18,20,22}, and to either an excess of ^{126}Xe , or a deficiency of ^{124}Xe , in solar xenon as a result of nuclear reactions^{20,23}. Eberhardt *et al.*¹⁶ have also suggested the possibility that fractionation might not be responsible for major differences in the isotopic composition of solar and terrestrial xenon, but that these two types of xenon might originate from different nucleosyntheses.

We have noted²⁴ that the xenon released from carbonaceous chondrites at $\approx 600^\circ\text{--}1,000^\circ\text{C}$ contains an excess of the proton-rich isotopes, $^{124}\text{--}^{128}\text{Xe}$, and the neutron-rich isotopes, $^{131}\text{--}^{136}\text{Xe}$. This observation suggests that meteoritic xenon consists of two isotopically distinct components whose presence cannot be explained by nuclear or fractionation processes within the meteorites. The meteoritic xenon component which is enriched in both the light and the heavy isotopes relative to ^{130}Xe was referred to as X, but the nature of the other meteoritic xenon component was not clearly defined. In this report the available data on xenon in carbonaceous chondrites, in lunar fines, and in the Earth's atmosphere are examined to seek a better understanding of the nature of these two components of meteoritic xenon.

General isotopic anomaly patterns of xenon

The isotopic composition of xenon in the three major reservoirs of xenon which have been studied; the Earth's atmosphere, meteorites, and lunar soil is presented in Table 1. The isotope ^{129}Xe is excluded because it is produced¹ in the early Solar System by the decay of primordial ^{129}I .

In Table 1, the nonradiogenic and nonspallogenic isotopes of xenon in lunar fines are used to represent the composition of solar xenon. The results from various laboratories are shown because estimates on the composition of xenon in lunar fines differ significantly between them, and there are differing opinions on the actual isotopic composition of xenon in the solar wind. These various estimates of solar xenon do however show a systematic relationship which suggests that they may be related by mass-dependent fractionation²⁰.

Two estimates for the composition of xenon in meteorites are represented in Table 1 by AVCC and WAVCC. The values for AVCC are an average of the estimate of trapped meteoritic xenon based on seven meteorites analysed by Marti⁴ and three

Table 1 Isotopic composition of solar, meteoritic and atmospheric xenon

Sample	^{124}Xe	^{126}Xe	^{128}Xe	^{130}Xe	^{131}Xe	^{132}Xe	^{134}Xe	^{136}Xe	Refs
Solar									
Lunar 16	2.99	2.78	51.0	100	494.7	600.6	220.1	176.0	19
Lunar Fines—10084.59	3.02	2.93	51.5	100	498.0	606.0	220.0	179.0	15
Lunar Fines—10084.48	2.94	2.55	50.8	100	496.0	608.0	224.2	182.6	17
Lunar Fines—SUCOR	2.89	2.63	50.9	100	499.0	606.6	225.2	182.7	18
Lunar Fines—10084.47	2.99	2.67	50.45	100	495.5	607.0	225.8	184.2	16
Lunar Fines—15601.64	2.73	2.61	50.6	100	501.2	613.5	229.4	187.1	20
Meteoritic									
AVCC	2.828	2.533	50.38	100	506.7	620.9	236.9	199.0	4, 5
WAVCC	—	—	50.82	100	506.3	617.9	235.5	197.2	This work
Atmospheric									
Air	2.353	2.206	47.03	100	519.1	659.1	255.9	217.4	25
Air	2.334	2.175	47.07	100	522.4	661.4	256.7	218.2	18

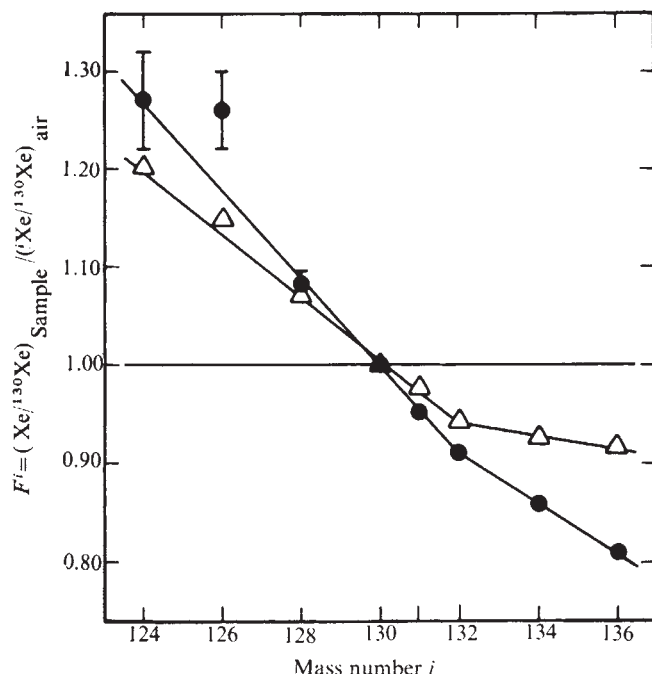


Fig. 1 A comparison of AVCC xenon^{4,5} (Δ) and solar xenon¹⁹ ($\bullet$) with xenon in the Earth's atmosphere²⁵. The break in the lines at ^{132}Xe indicates that neither of these two forms of extraterrestrial xenon can be related to atmospheric xenon by mass-dependent fractionation.

carbonaceous chondrites analysed by Eugster *et al.*⁵. The values shown for WAVCC represent a weighted average for approximately 75 analyses in different laboratories on a total of 35 carbonaceous and gas-rich meteorites, as listed in Table 5 of Mazor *et al.*²⁶. These results were weighted according to the xenon content of the meteorites. The abundances of the two light xenon isotopes, ^{124}Xe and ^{126}Xe , are not shown in WAVCC as there are no data on these isotopes for many of the analyses²⁶. The lower abundance of the heavy xenon isotopes in WAVCC than in AVCC indicates that the relative abundance of the heavy isotopes is lower in xenon-rich meteorites than in average carbonaceous chondrites. Atmospheric xenon is represented in Table 1 by the widely quoted analysis by Nier²⁵ and by a recent analysis by Podosek *et al.*¹⁸.

To compare the composition of xenon in these three reservoirs, we used the method of Canals *et al.*²⁷, in which the abundance of each isotope of mass number, i , is compared with its atmospheric abundance by the factor, F^i , defined as follows:

$$F^i = (X_e/^{130}X_e)_{\text{sample}} / (X_e/^{130}X_e)_{\text{air}} \quad (1)$$

The values of F^i for solar and AVCC xenon are plotted against mass number, i , in Fig. 1. For this comparison we have used Nier's value for atmospheric xenon²⁵ and Kaiser's value for solar xenon¹⁹. The latter was selected because it represents an extreme of the estimates for solar xenon and has been earlier depicted as solar xenon with the lowest fission component.

Several investigators¹⁴⁻¹⁷ have suggested that solar xenon and

AVCC xenon are identical, except for the presence of excess $^{132-136}\text{Xe}$ in the latter. Atmospheric xenon and AVCC xenon can be related by mass-dependent fractionation¹⁻⁵, except for the presence of excess $^{132-136}\text{Xe}$ in the latter. Thus, the view of solar and AVCC xenon expressed by these investigators¹⁴⁻¹⁷ suggests that atmospheric and solar xenon are directly related by fractionation. Kaiser¹⁹ has recently interpreted his results on xenon in Luna 16 as evidence that atmospheric xenon is, in fact, fractionated solar xenon. But, as can be seen in Fig. 1, even for the extreme estimate of solar xenon as given by Kaiser¹⁹, there is steeper fractionation across the light isotopes, $^{124-131}\text{Xe}$, than across the heavy isotopes, $^{132-136}\text{Xe}$. The break in the fractionation patterns for both AVCC and solar xenon at ^{132}Xe clearly shows that neither of these two reservoirs of extraterrestrial xenon can be related to atmospheric xenon by simple mass-dependent fractionation.

Component X and trapped meteoritic xenon

Reynolds and Turner⁶ have reported the presence of excess heavy xenon isotopes in the gas released from the Renazzo carbonaceous chondrite at 700°–1,000° C. Stepwise heating experiments on other carbonaceous chondrites in several laboratories⁷⁻¹³ confirmed this anomaly. We have reported²⁴ that the results of stepwise heating experiments showed an enrichment of ^{124}Xe in the same temperature fractions which contained excess ^{136}Xe . The correlation observed between light and heavy isotopes could not be explained by any of the previously proposed mechanisms, and we concluded that the observed anomaly pattern was caused by the release of an isotopically distinct component of xenon, referred to as component X, which had been incorporated into the meteorites.

Phinney's¹³ analyses for xenon released by stepwise heating of carbonaceous chondrites for the temperature fractions showing the largest enrichment of ^{136}Xe are listed in Table 2 together with the results of stepwise heating experiments discussed in our earlier report²⁴.

The correlation between the enrichment of the light isotopes, ^{124}Xe and ^{126}Xe , and the enrichment of the heaviest isotope, ^{136}Xe , in the xenon released by stepwise heating can be seen in Fig. 2, where $^{124}\text{Xe}/^{130}\text{Xe}$ and $^{126}\text{Xe}/^{130}\text{Xe}$ are plotted against $^{136}\text{Xe}/^{130}\text{Xe}$. Estimates of solar, atmospheric, and meteoritic xenon from Table 1 are shown for comparison. As can be seen from Fig. 2, the data of Phinney¹³ agree with the correlation reported earlier²⁴. The abundances of ^{124}Xe and ^{126}Xe have not been corrected for cosmic-ray induced spallation reactions, which may partially account for the scatter of data points from the correlation lines.

The dashed line in Fig. 2 shows the relationship expected from diffusive fractionation²⁸, and the failure of solar xenon to lie on the fractionation line passing through atmospheric xenon is understood in terms of the relationship shown in Fig. 1. The solid line connecting atmospheric and solar xenon defines the isotopic composition of xenon which would be produced by mixing components from these two xenon reservoirs. This 'mixing' line approximately parallels the fractionation relationship. If AVCC Xe really represents an average of the total xenon in meteorites, then the composition of trapped meteoritic xenon, when corrected for the presence of component X, would be

Table 2 Anomalous xenon released in stepwise heating of the carbonaceous chondrites

Sample	^{124}Xe	^{126}Xe	^{128}Xe	^{130}Xe	^{131}Xe	^{132}Xe	^{134}Xe	^{136}Xe	Refs
Renazzo 800° C	2.93 ± 0.05	2.57 ± 0.03	50.6 ± 0.4	100	505.8 ± 2.4	617.2 ± 1.9	243.3 ± 2.2	210.4 ± 1.5	6
Leoville 1,010° C	3.07 ± 0.09	2.66 ± 0.12	51.4 ± 0.5	100	511.3 ± 2.4	625.0 ± 3.9	250.0 ± 2.4	220.6 ± 2.3	11
Mokoia 700° C	3.43 ± 0.05	3.02 ± 0.06	51.9 ± 0.4	100	512.0 ± 2.5	625.0 ± 2.4	269.7 ± 1.3	244.8 ± 1.2	13
Mokoia (matrix) 600° C	3.15 ± 0.09	2.72 ± 0.09	52.7 ± 0.7	100	519.9 ± 6.8	633.3 ± 7.6	276.1 ± 3.6	248.9 ± 3.6	9
Mokoia 750° C	3.39 ± 0.06	2.90 ± 0.05	52.0 ± 0.8	100	514.1 ± 5.6	624.5 ± 5.6	278.6 ± 3.2	261.1 ± 2.0	12
Mokoia 800° C	3.44 ± 0.04	2.83 ± 0.04	52.2 ± 0.2	100	514.8 ± 1.8	628.9 ± 1.9	280.8 ± 1.0	263.6 ± 1.1	13
Allende 700° C	3.37 ± 0.10	2.93 ± 0.10	50.7 ± 0.5	100	516.3 ± 3.2	633.7 ± 3.5	289.2 ± 2.7	273.6 ± 3.0	13
Allende 1,000° C	3.39 ± 0.14	2.77 ± 0.09	54.5 ± 0.9	100	513.0 ± 6.1	624.9 ± 8.6	291.2 ± 3.9	282.5 ± 2.4	12
Allende 600° C	3.42 ± 0.09	3.02 ± 0.12	52.1 ± 0.9	100	522.7 ± 5.6	643.1 ± 6.2	300.8 ± 5.0	290.0 ± 3.4	13
Allende 800° C	3.62 ± 0.13	2.84 ± 0.09	52.1 ± 1.5	100	518.6 ± 11.9	641.0 ± 14.3	305.1 ± 7.0	299.4 ± 5.4	12

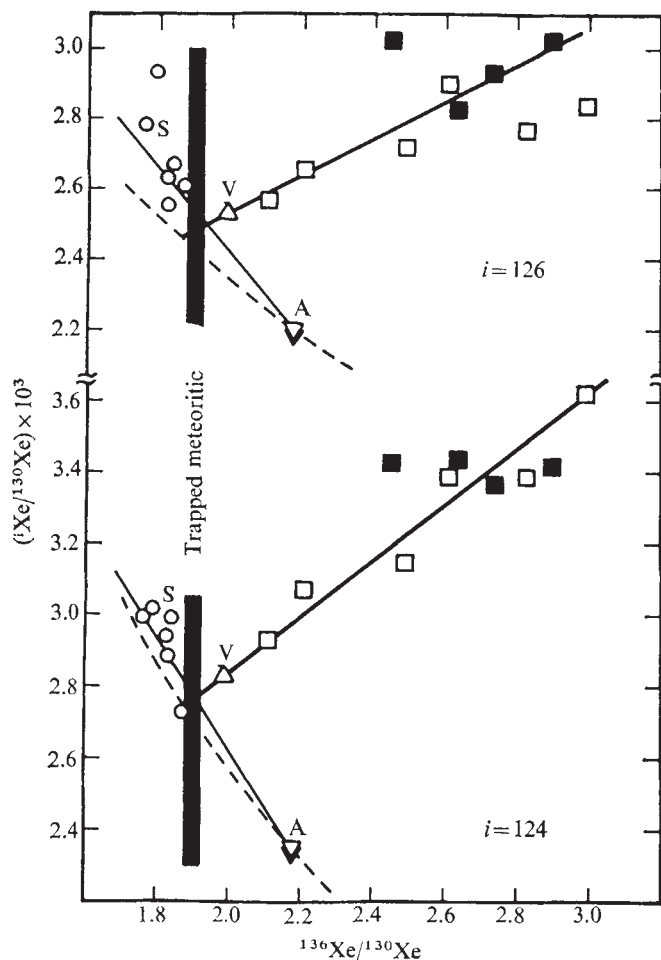


Fig. 2 The correlations of $^{124}\text{Xe}/^{130}\text{Xe}$ and $^{126}\text{Xe}/^{130}\text{Xe}$ with $^{136}\text{Xe}/^{130}\text{Xe}$ in the xenon released by stepwise heating of carbonaceous chondrites. The correlations between these xenon isotope ratios are shown by the heavy lines. ■ Represents recent stepwise heating data by Phinney¹³. The symbols S, V, A are shown above solar, AVCC, and atmospheric xenon, respectively. The dashed line passing through atmospheric xenon shows the isotope compositions expected from diffusive fractionation, and the solid line passing through atmospheric and solar xenon show the isotopic compositions expected from mixing these two types of xenon.

expected to lie to the left of AVCC Xe on the correlation lines shown in Fig. 2. Likewise, the subtraction of component X from the xenon released at each extraction temperature might leave a common trapped meteoritic xenon component. Since the other two known reservoirs of xenon are solar and atmospheric, a suitable choice for trapped meteoritic xenon can be characterised by the intersections of the correlation lines with the 'mixing' line or the fractionation line. The darkened column labelled trapped meteoritic in Fig. 2 represents these intersections. Until the isotopic composition of solar xenon is sufficiently well established to indicate clearly the relationship between solar and atmospheric xenon, a decision on which intersection represents 'trapped meteoritic' xenon would be arbitrary. Since these three reservoirs of xenon are probably genetically related, though not necessarily by a diffusive fractionation relationship, we will postulate a simple linear relationship between atmospheric and solar xenon to see if meteoritic xenon can be resolved into a mixture of component X and a self-consistent composition for trapped meteoritic xenon.

The observed correlations between the other nonradiogenic xenon isotopes released in the stepwise heating of carbonaceous chondrites are shown in Fig. 3, together with the 'mixing' line between solar and atmospheric xenon. All the xenon isotope data from Table 1 and Table 2 for $i = 128$ –136 are shown in Fig. 3, except for the ratio, $^{128}\text{Xe}/^{130}\text{Xe}$, in the xenon released

from the Allende meteorite at 1,000° C. The unusually high value for this ratio, indicated by the arrow in Fig. 3, is attributed to neutron-capture reactions on ^{127}I in this meteorite. Fireman *et al.*²⁹ and Manuel *et al.*¹² have reported an excess of certain noble gas isotopes in Allende as a result of neutron-capture reactions on halogens.

From Fig. 3 it appears that the points of intersection of the correlation lines and the 'mixing' lines define a self-consistent composition for trapped meteoritic xenon, and this composition is also consistent with the trapped xenon shown in Fig. 2. The isotopic composition of trapped meteoritic xenon defined by these intersections is $^{124}\text{Xe}:^{126}\text{Xe}:^{128}\text{Xe}:^{130}\text{Xe}:^{131}\text{Xe}:^{132}\text{Xe}:^{134}\text{Xe}:^{136}\text{Xe} = 0.0276:0.0248:0.501:1.00:5.04:6.19:2.31:1.90$. The scatter of data points about the correlation lines in Fig. 3 may be due to variations in the compositions of trapped meteoritic xenon. If the trapped meteoritic xenon lies along the 'mixing' line, as would be expected if meteoritic xenon has been acted on by the same process responsible for the relationship between atmospheric and solar xenon, then this might account for the smallest scatter of data points along the correlation line for $i = 134$. For this isotope, the slope of the 'mixing' line and the slope of the correlation line are most nearly equal.

It should be noted from Fig. 3 that, relative to ^{130}Xe , all the other xenon isotopes are enriched in component X when compared with their abundance in trapped meteoritic xenon. We have considered²⁴ possible origins for component X earlier and shall now discuss the relationship between AVCC xenon, trapped meteoritic xenon, and component X.

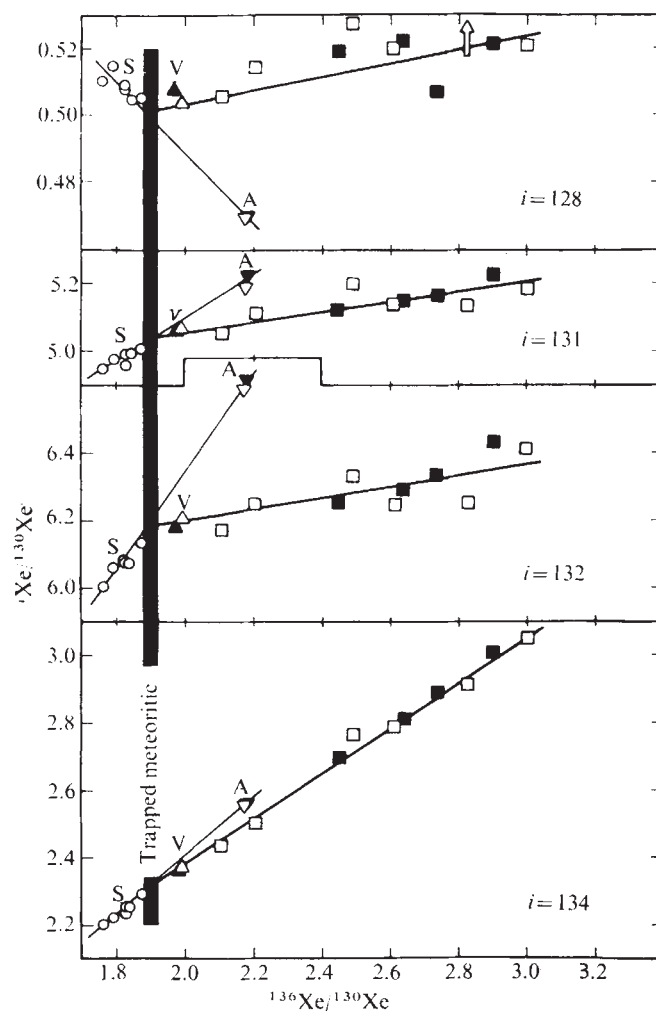


Fig. 3 The correlation of other xenon isotope ratios with $^{136}\text{Xe}/^{130}\text{Xe}$ in the xenon released by stepwise heating of carbonaceous chondrites. The lines and symbols are as defined for Fig. 2 except that both AVCC and WAVCC are shown below the symbol V.

AVCC xenon

Mazor *et al.*²⁶ have shown the results of approximately 75 analyses for meteoritic xenon from several laboratories. These results are for total xenon and do not include data on ^{124}Xe and ^{126}Xe for most of the meteorites. No data on ^{130}Xe are given for four of the analyses. Thus, it is not possible to determine whether the total xenon in these meteorites shows a correlation between an enrichment of the light isotopes and an enrichment of the heavy isotopes, as shown in Fig. 2 for stepwise heating experiments.

But, even in total melt experiments, we find variations in the relative abundances of the heavy xenon isotopes as observed for stepwise heating experiments. This correlation of excess ^{136}Xe with excess ^{134}Xe in about 70 total melt analyses of the xenon in 35 different meteorites is shown in Fig. 4. The dashed lines indicate the area of uncertainty for the correlation which would result if points on the solid correlation line had an uncertainty of $\pm 1\%$. This degree of uncertainty is typical of the xenon isotope measurements, and this is shown for a symbolic data

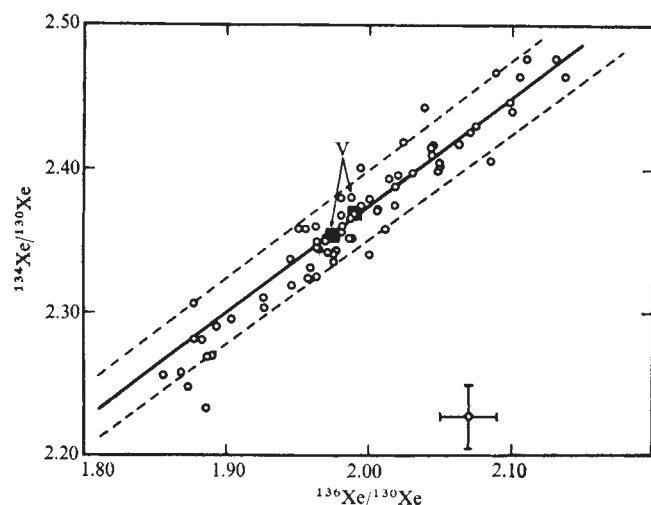


Fig. 4 The correlation of $^{134}\text{Xe}/^{130}\text{Xe}$ with $^{136}\text{Xe}/^{130}\text{Xe}$ in the total xenon of meteorites. Data are from a compilation by Mazor *et al.*²⁶. The darkened squares, labelled, V, show the isotopic composition of AVCC and WAVCC xenon. Typical error limits of $\pm 1\%$ are shown in the lower right corner.

point in the lower right of Fig. 2. The isotopic compositions given in Table 1 for AVCC and WAVCC are shown in Fig. 4. The fact that these lie near the middle of the graph, which is heavily populated with data points representing total meteoritic xenon, demonstrates that AVCC and WAVCC satisfactorily describe the average isotopic abundances of ^{130}Xe , ^{134}Xe and ^{136}Xe in total meteoritic xenon.

The correlation of excess ^{134}Xe with excess ^{136}Xe for total meteoritic xenon, in the manner observed for xenon released by stepwise heating experiments, suggests that the same mechanism may account for both the isotopic variations in different temperature fractions of a meteorite and the isotopic variations in the total xenon from different meteorites. Recently Takaoka³⁰ reiterated the view that correlations between isotope ratios for ^{128}Xe , ^{130}Xe , ^{131}Xe , ^{132}Xe , ^{134}Xe and ^{136}Xe in the total xenon of different carbonaceous chondrites can be accounted for by the carbonaceous chondrite fission (CCF) hypothesis⁶. Takaoka did not, however, consider the isotopic abundances of ^{124}Xe and ^{126}Xe . Although the correlation shown in Fig. 4 is consistent with both the CCF hypothesis and the component X hypothesis, we assume that the correlation results from variations in the relative amounts of component X for the following reason. This same mechanism can also account for isotopic correlations observed in stepwise heating experiments, whereas the CCF hypothesis fails to account for the correlations of excess ^{124}Xe and excess ^{126}Xe with excess ^{136}Xe , as shown in Fig. 2.

Another mechanism which may contribute to differences in the isotopic composition of atmospheric, solar and AVCC xenon has been noted by Kuroda³¹. He suggests that neutron-capture reactions in the interior of the sun during the deuterium-burning stage may have enriched solar xenon in the intermediate mass isotopes, $^{128}\text{--}^{132}\text{Xe}$. Xenon which had been isolated from the sun before the onset of deuterium burning would thus appear to be enriched in the lighter and heavier isotopes, a pattern observed in component X. Fractional differences between the abundances of ^{130}Xe in component X and other sources are, however, much larger than those which Kuroda³¹ attributes to solar nuclear reactions.

From the range of $^{136}\text{Xe}/^{130}\text{Xe}$ values shown in Figs 3 and 4 we conclude that for total melt experiments the $^{136}\text{Xe}/^{130}\text{Xe}$ ratio may differ from the $^{136}\text{Xe}/^{130}\text{Xe}$ ratio in trapped meteoritic xenon by $\approx 0\text{--}11\%$ as a result of variations in the relative amounts of component X, whereas stepwise heating experiments partially separate these two components of meteoritic xenon to yield $^{136}\text{Xe}/^{130}\text{Xe}$ ratios that are $\approx 0\text{--}58\%$ higher than the $^{136}\text{Xe}/^{130}\text{Xe}$ ratio in trapped meteoritic xenon. AVCC xenon represents the isotopic composition for the average observed mixture of trapped meteoritic xenon and component X.

This investigation was supported by grants from NASA and NSF.

Received August 17; revised December 5, 1973.

- ¹ Reynolds, J. H., *Phys. Rev. Lett.*, **4**, 8 (1960).
- ² Reynolds, J. H., *Phys. Rev. Lett.*, **4**, 351 (1960).
- ³ Kruppenacher, D., Merrihue, C. M., Pepin, R. O., and Reynolds, J. H., *Geochim. cosmochim. Acta*, **26**, 231 (1962).
- ⁴ Marti, K., *Earth planet. Sci. Lett.*, **3**, 243 (1967).
- ⁵ Eugster, O., Eberhardt, P., and Geiss, J., *Earth planet. Sci. Lett.*, **3**, 249 (1967).
- ⁶ Reynolds, J. H., and Turner, G., *J. geophys. Res.*, **69**, 3263 (1964).
- ⁷ Turner, G., *J. geophys. Res.*, **70**, 5433 (1965).
- ⁸ Funk, H., Podosek, F., and Rowe, M. W., *Geochim. cosmochim. Acta*, **31**, 1721 (1967).
- ⁹ Rowe, M. W., *Geochim. cosmochim. Acta*, **32**, 1317 (1968).
- ¹⁰ Pepin, R. O., *Origin and Distribution of the Elements* (edit. by Ahrens, L. H.), 379 (Pergamon Press, Oxford and New York, 1968).
- ¹¹ Manuel, O. K., Wright, R. J., Miller, D. K., and Kuroda, P. K., *J. geophys. Res.*, **75**, 5693 (1970).
- ¹² Manuel, O. K., Wright, R. J., Miller, D. K., and Kuroda, P. K., *Geochim. cosmochim. Acta*, **36**, 961 (1972).
- ¹³ Phinney, D. L., Dissertation, Univ. Minnesota (1971).
- ¹⁴ Marti, K., *Science*, **166**, 1263 (1969).
- ¹⁵ Hohenberg, C. M., Davis, P. K., Kaiser, W. A., Lewis, R. S., and Reynolds, J. H., *Geochim. cosmochim. Acta*, Suppl. 1, **2**, 1283 (1970).
- ¹⁶ Eberhardt, P., Geiss, J., Graf, H., Grögler, N., Krähenbühl, U., Schwaller, H., Schwarzmüller, J., and Stettler, A., *Geochim. cosmochim. Acta*, Suppl. 1, **2**, 1037 (1970).
- ¹⁷ Pepin, R. O., Nyquist, L. E., Phinney, D., and Black, D. C., *Geochim. cosmochim. Acta*, Suppl. 1, **2**, 1435 (1970).
- ¹⁸ Podosek, F. A., Huneke, J. C., Burnett, D. S., and Wasserburg, G. J., *Earth planet. Sci. Lett.*, **10**, 199 (1971).
- ¹⁹ Kaiser, W. A., *Earth planet. Sci. Lett.*, **13**, 387 (1972).
- ²⁰ Srinivasan, B., Hennecke, E. W., Sinclair, D. E., and Manuel, O. K., *Geochim. cosmochim. Acta*, Suppl. 3, **2**, 1927 (1972).
- ²¹ Kuroda, P. K., and Manuel, O. K., *Nature*, **227**, 1113 (1970).
- ²² Boulos, M. S., and Manuel, O. K., *Science*, **174**, 1334 (1971).
- ²³ Hennecke, E. W., and Manuel, O. K., *Z. Naturforsch.*, **26a**, 1980 (1971).
- ²⁴ Manuel, O. K., Hennecke, E. W., and Sabu, D. D., *Nature*, **240**, 99 (1972).
- ²⁵ Nier, A. O., *Phys. Rev.*, **79**, 450 (1950).
- ²⁶ Mazor, E., Heymann, D., and Anders, E., *Geochim. cosmochim. Acta*, **34**, 781 (1970).
- ²⁷ Canals, R. A., Alexander, E. C. jun., and Manuel, O. K., *J. geophys. Res.*, **73**, 3331 (1968).
- ²⁸ Aston, F. W., *Mass-Spectra and Isotopes*, 219 (Edward Arnold and Co., London, 1933).
- ²⁹ Fireman, E. L., Defelice, J., and Norton, E., *Geochim. cosmochim. Acta*, **34**, 873 (1970).
- ³⁰ Takaoka, N., *Mass Spectroscopy*, **20**, 287 (1972).
- ³¹ Kuroda, P. K., *Nature*, **230**, 40 (1971).

Gas streaming in 2U0900-40 and Cyg X-1

D. T. Wickramasinghe & M. S. Bessell

Mount Stromlo and Siding Spring Observatory, Research School of Physical Sciences, The Australian National University, Australia

Published photometric data on 2U0900-40 and Cyg X-1 are interpreted as evidence of gas streaming in these systems. The location and extent of the gas streams indicate that they originate from the accretion disk and extend to the far side of the primary. We suggest that large scale mass loss is occurring from the outer regions of the accretion disk as a result of radiative acceleration by a high luminosity component of soft X rays ($\epsilon \lesssim 1$ keV), and offer a possible interpretation of the radio observations of Cyg X-1.

Several X-ray sources have been identified with binary systems, the light curves of which show two maxima and two minima per orbital period¹⁻⁵ characteristic of 'ellipsoidal' variables. The recently reported failure to detect the secondary minimum of Cyg X-1 (refs 6-7) has, however, cast some doubt on the conventional interpretation of the light curves in terms of gravity and limb darkening effects of a tidally distorted star. Here we suggest a possible interpretation of both the Cyg X-1 observations, and the recently published photometric data on HD77581 which indicates variability of the light curve following secondary minimum, in terms of a model invoking gas streaming on the 'far side' of the primary as viewed from the X-ray source. It is suggested that the variable radio emission observed in Cyg X-1 could originate in an expanding envelope surrounding the binary system.

Evidence of gas streaming

Wickramasinghe and Whelan⁸ and Hutchings⁹ have attempted to analyse the light curve of HD77581 in the framework of a model in which the primary is in near contact with its inner critical Roche lobe but were unable to obtain a detailed fit near secondary minimum. Agreement between theory and observations was found to be better if an eccentric orbit is assumed⁹. This interpretation, we feel, can now be ruled out on the basis of the detailed observations recently published by Jones and Liller¹⁰ which we present in Fig. 1. (Phase $\phi = 0$ corresponds to X-ray eclipse—also secondary minimum.) The solid line corresponds to the theoretical model computed by Wickramasinghe and Whelan⁸ assuming a circular orbit. The failure to recover full brightness following X-ray eclipse on March 5, 1973, as shown by these observations is strongly suggestive of obscuration of the primary between phases $\phi = 0$ and $\phi = 0.5$. There is also some evidence for a similar effect on a smaller scale over the same phase in the previous cycle and for even smaller effects at other phases.

Any outflow of matter from the primary (defined as the more massive component) through the inner Lagrangian point L_1 is likely to be subsonic and therefore cannot give rise to a gas stream which extends to the far side of the star. One of us¹¹ has recently pointed out the possibility of radiative acceleration of material at the outer edges of the accretion disk by a high luminosity component of soft X rays (of energy $\epsilon < 1$ keV). In this case, it is possible that some of the radiatively accelerated material could give rise to an optically thick gas stream which falls back on the surface of the primary as illustrated in Fig. 2. Alternatively, the material could be accelerated to velocities in excess of the escape velocity resulting in mass loss from the system. Either way the flow is expected to be concentrated in the orbital plane with the density being highest near the stellar

surface. The observed anisotropy of the flow with respect to the line of centres is due to the initial angular momentum of the accelerated material. We note that in addition to the diminution in light, the (U-B) and (B-V) colours are expected to become bluer during the equatorial obscuration, since only the hotter polar regions will be visible. There is some evidence for this effect in the observational data (Fig. 1).

An optically thick region of thickness $\Delta h_{st} \sim 1/10 R_s$ (normal to the orbital plane) where $R_s \sim 30 R_\odot$ is the stellar radius, would suffice to explain the observed (0.08 mag) diminution in light at phase $\phi \approx 0.25$. Adopting a value of $5 R_\odot$ for the radial thickness of the effective obscuring region, we estimate an electron number density $n_e \gtrsim 10^{13} \text{ cm}^{-3}$ assuming that electron scattering is the dominant opacity source. A lower density may be appropriate if the observed extinction occurs over an extended region in the orbital plane.

A lower limit to the rate of mass transfer in the gas stream is given by $m_H \Delta h_{st} V_g / \sigma_T$ where σ_T , m_H , and V_g are the electron scattering cross section, the mass of the hydrogen atom and the stream velocity respectively. Assuming that V_g is near the escape velocity the above expression yields $\sim 10^{-7} M_\odot \text{ yr}^{-1}$. The rate of mass loss from the entire disk is thus likely to be at least $10^{-6} M_\odot \text{ yr}^{-1}$ and could be considerably greater.

Such a high rate of mass ejection from the accretion disk of the secondary apparently occurs only during some cycles although there is probably continuous outflow at much lower densities. This, together with the fact that the observed X-ray luminosity ($\sim 10^{37} \text{ erg s}^{-1}$) requires a steady accretion rate of 10^{-8} – $10^{-9} M_\odot \text{ yr}^{-1}$ (ref. 12) for a neutron star or black hole secondary, suggests that a local enhancement in the rate of mass transfer from the primary through L_1 could trigger an increase in the rate of radiation-pressure driven mass loss from the outer edges of the disk with little change in the X-ray luminosity. In this connection it should be noted that there is considerable observational and theoretical evidence, which supports the hypothesis that mass transfer through L_1 is the dominant mechanism of mass exchange in X-ray binaries with a supergiant component¹¹.

We note that the theoretical light curve near secondary

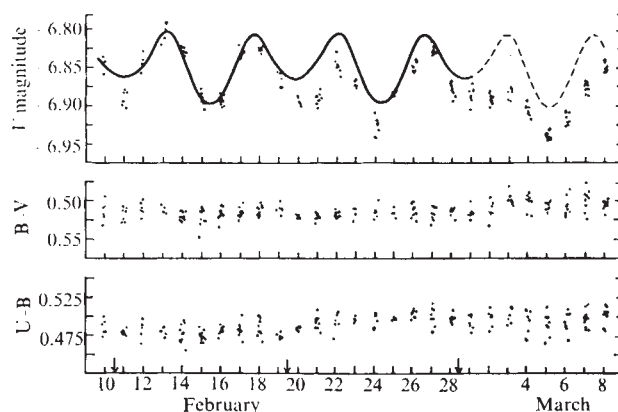


Fig. 1 The V, (B-V) and (U-B) observations of Jones and Liller¹⁰. The arrow corresponds to X-ray eclipse. The continuous curve is the zero eccentricity model of Wickramasinghe and Whelan⁸ for an orbital inclination $i \sim 90^\circ$ and a mass ratio $q \sim 0.09$.

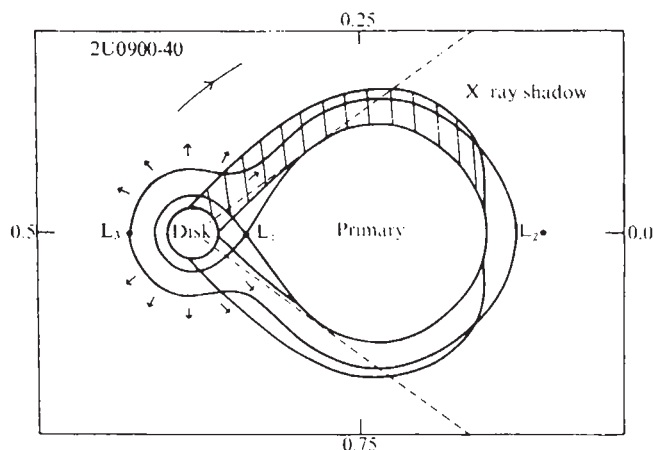


Fig. 2 A schematic representation of a possible flow pattern in the orbital plane (neglecting rotation) for a mass ratio $q \sim 0.09$. The Roche equipotentials through the Lagrangian points L_1 and L_2 have been drawn to scale. The hatched area represents the probable location of the gas streams causing maximum obscuration.

minimum is shallower than the observed light curve even during the first cycle. It is plausible that the continuous low density outflow from the disk envisaged earlier could give rise to obscuration mainly near secondary minimum when the material in the region of the X-ray shadow lies between the observer and the primary⁸. The possibility of detecting the gas streams spectroscopically should also be greatest at these phases. On this interpretation the gas streams nearest the stellar surface are required to fall back on the far side of the primary rather than be accelerated to velocities in excess of the escape velocity.

The possibility that radiative acceleration by soft X rays may prevent the development of an appreciable stellar wind from the stellar surface facing the X-ray source has recently been discussed by one of us¹¹. It is accordingly interesting that the estimated mass flux in the stream indicates that rates of mass loss in excess of normal stellar wind values may be possible from the accretion disk during some cycles. The flow patterns in the two cases are however likely to be quite distinct as indicated earlier. A wind from the accretion disk would be concentrated largely in the orbital plane and will tend to avoid the region of the X-ray shadow.

Walker⁸ has observed the light curve of HD226868 over several cycles during the period May to June, 1973 and reports regular primary minima with variable secondary minima. The secondary minimum is reported to be completely filled in during some cycles. This result has also been confirmed by Martynov⁷.

An explanation similar to that offered for HD77581 is possible if it is noted that this system is viewed at a high orbital inclination as is evident from the absence of an X-ray occultation. A sufficiently extensive scattering region in the orbital plane at the far side of the primary could thus act as an additional source of light at secondary minimum.

We note that the mass ratio $q (= M_2/M_1) \sim 0.6$ of the Cyg X-1-HD226868 system¹³ is considerably larger than that of the 2U0900-40-HD77581 system⁸ (~ 0.09) where M_1 and M_2 are the masses of the primary and secondary respectively. The angular momentum problem is accordingly not as severe, and provided the initial acceleration of disk material is possible (for example by radiation pressure) gas streams may interact at the far side of the primary and form an accretion shock front which could serve as an effective scattering region (Fig. 3). The exceptionally large scatter in the light curve⁵ indicates that gas streams may contribute to the light at all phases.

It is evident that the volume of gas responsible for the filling in of the secondary minimum must be situated sufficiently close to the primary so that it may be eclipsed at the appropriate phases (~ 0.5). A mean distance of $\sim 1.5 R_s$ from the centre

of the primary may thus be appropriate. (We note that the orbital inclination i must be $< 90^\circ$ for the present model to be viable suggesting that values of i corresponding to grazing eclipse of the secondary may be required.) An order of magnitude estimate of the total number of electrons required to fill in the secondary minimum (of depth ~ 0.06 min) can be obtained from the equation $\sigma_T N_e W / \pi R_s^2 \sim 0.06$ where W is the geometrical dilution factor. Setting $W \sim 1/10$, $R_s \sim 10^{12}$ cm we obtain $N_e \sim 10^{48-49}$. Assuming further that the gas occupies a volume 10^{36} cm³ (1/10 stellar volume)—a plausible value from the eclipse point of view—we obtain an electron number density $n_e \sim 10^{12-13}$ cm⁻³. The scattering region is thus likely to be optically thin ($\tau_{es} \lesssim 1$) and could give rise to a detectable polarisation measure suggesting a possible test of the model. Further consequences of the proposed model should be noted.

(a) Accretion of matter on to the far side of the primary may result in the heating up of the stellar atmosphere. Assuming $M_1 \sim 20 M_\odot$ and a terminal velocity of the order of the escape velocity, we estimate a 10% increase in effective temperature for accretion rates $\sim 10^{-3} M_\odot \text{ yr}^{-1}$. The observed brightening of the star near secondary minimum could be the result of a general heating effect or a localised hot spot. Simultaneous spectroscopic and photometric observations at the appropriate phases would help eliminate this possibility. We note that on the scattering model the colours should remain almost unchanged when the secondary minimum is filled in.

(b) If we assume that the scattering material is in the region of the shock, the temperature of the gas is likely to be $\sim 10^7$ K. We estimate that the infrared contribution due to free-free emission should yield an excess ~ 0.5 mag in (K-M) near secondary minimum if $N_e \sim 10^{49}$. The optical contribution due to free-free emission is 0.1% of the stellar contribution and can be neglected.

(c) Cyg X-1 was observed as a weak radio source ($2 \times 10^{-28} \text{ W M}^{-2} \text{ Hz}^{-1}$ at 1415 MHz) in April 1971 (ref. 14). The source has a nearly flat radio spectrum in the frequency range 1,415–10,630 MHz and was not detected in surveys made before this date¹⁵. We note that free-free emission from an expanding envelope surrounding the binary system could result in an appreciable radio luminosity if the emitting region is optically thin to free-free absorption and is maintained at a high degree of ionisation. The former condition requires $n_e^2 L \lesssim 2 \times 10^{25}$ (assuming a temperature $T \sim 10^4$ K characteristic of HII regions) where L is the linear dimension of the emitting region. From the observed radio flux at 1,415 MHz we obtain $n_e^2 L^3 \sim 5 \times 10^{58}$. An extended region with $L \sim 5 \times 10^{16}$ cm and $n_e \sim 2 \times 10^4 \text{ cm}^{-3}$ would satisfy the above conditions. Whether the gas can remain sufficiently ionised at distances $\sim 10^{16}$ cm from the binary system is difficult to estimate without a detailed model for the outflow.

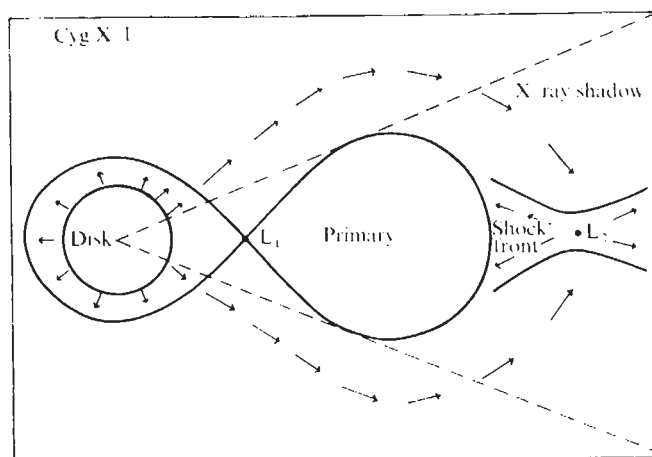


Fig. 3 A schematic representation of a possible flow pattern in the orbital plane (neglecting rotation) for a mass ratio $q \sim 0.6$. The location of the accretion shock front is also indicated.

We note, however, that a Stromgren sphere surrounding such a star would have a dimension $\sim 10^{17}$ cm (ref. 16) if the particle density is as high as $\sim 2 \times 10^4 \text{ cm}^{-3}$ indicating that our suggestion is plausible.

Since the details of the outflow from the accretion disk are not known it is not clear at present whether velocities in excess of the escape velocity can be achieved as a result of radiative acceleration by soft X rays. Preliminary estimates indicate that continued radiative acceleration is more likely to be achieved by the stellar ultraviolet radiation. Mass loss from the system may thus occur in the form of an intense 'stellar wind', the material of which originates from the accretion disk. Until the details of the model are worked out, our suggested interpretation of the Cyg X-1 observations must be considered as tentative.

Our conclusions may be summarised as follows:

(1) The observed variations in the shape of the light curve of HD77581 following X-ray minimum can be interpreted in terms of a model invoking gas streaming. The location and extent of the gas streams indicate that they originate from the accretion disk, possibly as a result of radiative acceleration. The variability of the gas streams is attributed to local changes in the rate of mass transfer through the inner Lagrangian point, which in turn effects the rate of mass loss from the disk.

(2) The Cyg X-1 observations can be interpreted in terms of a scattering region of volume 10^{36} cm^3 and electron density $n_e \sim 10^{12-13} \text{ cm}^{-3}$ situated at a distance of ~ 1.5 stellar radii from the primary. The model predicts a detectable polarisation measure and an infrared excess of ~ 0.5 mag in K-M near X-ray eclipse. The observed variable radio emission could originate as a result of free-free emission from the optically

thin regions of an expanding envelope surrounding the binary system.

(3) In view of the possible importance of gas streaming in 2U0900-40 and Cyg X-1, the determination of orbital eccentricities from the light curves must be viewed with caution. The interpretation of the radial velocity observations of these systems could also be complicated by gas streaming effects at certain phases.

We thank Dr A. R. Hyland for discussions and the referee for his comments.

Received March 6; revised April 23, 1974.

- ¹ Vidal, N. V., Wickramasinghe, D. T., and Peterson, B. A., *Astrophys. J. Lett.*, **182**, L77 (1973).
- ² Jones, C., and Liller, W., *Astrophys. J. Lett.*, **184**, L65 (1973).
- ³ Jones, C., Forman, W., and Liller, W., *Astrophys. J. Lett.*, **182**, L109 (1973).
- ⁴ Liller, W., *Astrophys. J. Lett.*, **184**, L37 (1973).
- ⁵ Walker, E. N., *Mon. Not. R. astr. Soc.*, **160**, 13P (1972).
- ⁶ Walker, E. N., *IAUC*, 2551 (1973).
- ⁷ Martynov, D. Ya., *IAUC*, 2575 (1973).
- ⁸ Wickramasinghe, D. T., and Whelan, J., *Mon. Not. R. astr. Soc.*, **167**, 21P (1974).
- ⁹ Hutchings, J., *Astrophys. J.*, **179**, 869 (1974).
- ¹⁰ Jones, C., and Liller, W., *Astrophys. J. Lett.*, **184**, L121 (1973).
- ¹¹ Wickramasinghe, D. T., *Mon. Not. R. astr. Soc.* (1974).
- ¹² Shakura, N. I., and Sunyaev, R. A., *Astr. Astrophys.*, **24**, 337 (1973).
- ¹³ Hutchings, J., Crampton, D., Glaspey, J., Walker, G. A. H., *Astrophys. J.*, **182**, 549 (1973).
- ¹⁴ Braes, L. L. E., and Miley, G. K., *Nature*, **232**, 246 (1971).
- ¹⁵ Hjellming, R. M., Wade, C. M., Hughes, V. A., and Woodsworth, A., *Nature*, **234**, 138 (1971).
- ¹⁶ Stromgren, B., *Astrophys. J.*, **89**, 526 (1939).

DNA polymerase activity from two temperature-sensitive mutants of Rous sarcoma virus is thermolabile

Inder M. Verma,[†] William S. Mason,^{*‡} Stanley D. Drost & David Baltimore

Department of Biology and Center for Cancer Research, Massachusetts Institute of Technology,
77 Massachusetts Avenue, Cambridge, Massachusetts 02139

Temperature sensitivity of growth and transformation by certain early Rous sarcoma virus mutants correlates with thermolability of their virion-associated DNA polymerase. The enzyme is therefore encoded by viral RNA and is required for provirus synthesis.

Two temperature sensitive mutants of Rous sarcoma virus (RSV) have been isolated which have defects very early in their growth cycle (ref. 1 and W. S. M., R. R. Friis, M. Linial and P. K. Vogt, in preparation). They are called *ts335* and *ts337*. Their temperature sensitive function is necessary for the initiation but not the maintenance of both viral replication and cell transformation (ref. 2 and in preparation). The DNA poly-

merase activity in the mutant virions appears to be temperature sensitive when assayed with either endogenous or exogenous templates (ref. 2 and in preparation). (According to the system for virus nomenclature proposed by Vogt, Weiss and Hanafusa³ these viruses are designated as *tsLA335PR-C* and *tsLA337PR-C*.)

We have purified the DNA polymerase from both *ts335* and *ts337* and compared the activities of the purified enzymes with those of the DNA polymerase of wild type RSV. The three known activities of the avian RNA tumour virus DNA polymerase have been studied: RNA-directed DNA synthesis, DNA-directed DNA synthesis, and ribonuclease H (which selectively degrades the RNA portion of a DNA-RN hybrid⁴).

Thermolabile enzymes

DNA polymerase activity of detergent-treated *ts335* and wild type RSV virions at 34°, 37° and 40.5° C was measured both in the endogenous reaction and after addition of an exogenous template · primer. The endogenous activity of *ts335* was slightly lower at 40.5° C than at 34° C, while the activity of the RSV wild type increased two-fold through this temperature range (Table 1). With poly(C) · oligo(dG) as an added template · primer⁵, *ts335* DNA polymerase incorporated about one-third

^{*}Department of Microbiology, University of Southern California, School of Medicine, 2025 Zonal Avenue, Los Angeles, California 90033

[†]Present Address: The Salk Institute, PO Box 1809, San Diego, California 92112.

[‡]Present Address: Institute for Cancer Research, 7701 Burholme Avenue, Philadelphia, Pennsylvania 19111.

Table 1 DNA polymerase activity associated with purified virions of *ts335* and RSV wild type

Temperature (°C)	Endogenous activity (pmol ³ H-dAMP incorporated)			Exogenous activity with poly(C) · oligo(dG) (pmol ³ H-dGMP incorporated)		
	<i>ts335</i>	RSV(WT)	Ratio of RSV(WT): <i>ts335</i>	<i>ts335</i>	RSV(WT)	RSV(WT): <i>ts335</i>
34	40	28	0.7	239	315	1.32
37	35	41	1.17	203	375	1.85
40.5	33	61	1.84	68	406	5.97

The wild type sarcoma virus was the Prague strain (RSV(WT)); *ts335* was isolated as described¹. Both viruses belong to subgroup C. The virus stocks were grown at 35° C and purified as before². Protein concentrations were determined using the procedure of Lowry *et al.*²³ with bovine serum albumin as a calibration standard. All oligomers used as primers were 12 to 18 nucleotides long.

For the endogenous reaction, assays of heat lability were performed by incubating the purified virions in 0.1 ml of reaction mixture containing 50 mM Tris-HCl, pH 8.3, 5 mM dithiothreitol, 6 mM magnesium acetate, 60 mM NaCl, 60 nmol each dTTP, dCTP, and dGTP, 20 nmol ³H-dATP (specific activity 100 c.p.m. pmol⁻¹), Nonidet P-40 to a final concentration of 0.2%, and 12 µg of viral protein. The reaction mixtures were incubated separately at 34° C, 37° C, or 40.5° C for 90 min and radioactivity in acid insoluble material was detected as described before²⁴. To assay exogenous activity, purified virions containing 2.4 µg of protein in 0.05 ml of reaction mixture I (100 mM Tris-HCl, pH 8.3, 10 mM dithiothreitol, 12 mM Mg acetate, 60 mM NaCl, and 0.2% Nonidet P-40) were incubated at 34° C, 37° C, or 40.5° C for 10 min. After preincubation, to each reaction mixture was added 0.05 ml of reaction mixture II (20 nmol ³H-dGTP, (100 c.p.m. pmol⁻¹), 2.0 µg poly(C) and 0.5 µg oligo(dG). The reaction mixture was further incubated for 15 min at 34° C, 37° C or 40.5° C and acid-precipitable radioactivity monitored as described before²⁴.

the amount of ³H-dGMP at 40.5° C as at 34° C, while incorporation by wild type RSV DNA polymerase was increased at 40.5° C (Table 1). Thus, in disrupted virions, the *ts335* DNA polymerase was more sensitive to thermal inactivation than the wild type RSV DNA polymerase, in agreement with previous data².

DNA polymerase was purified from wild type RSV and from the two mutants by standard procedures⁶. Although the enzymes

from the mutants were inactivated more extensively than the wild type enzyme during purification and subsequent storage at -70° C in 20% glycerol, the three enzymes behaved indistinguishably during ion-exchange chromatography on DEAE-Sephadex and phosphocellulose.

Electrophoresis on SDS-containing polyacrylamide gels followed by staining with Coomassie blue⁷, showed that the purified DNA polymerases from *ts335*, *ts337* and wild type RSV had two polypeptide bands of average molecular weight 88,000 and 56,000. The subunits were in equimolar ratio. In some samples there occasionally appeared a faint band at about 64,000 which we assume to be a contaminant. The purified DNA polymerase from avian myeloblastosis virus (AMV) was not distinguishable from the RSV enzymes. These values for the molecular weights of the polypeptides of the DNA polymerase are somewhat lower than those reported by others⁸⁻¹⁰.

The heat-inactivation kinetics of the ability of the purified enzymes to copy ribohomopolymers were assayed using poly(C) · oligo(dG) and poly(A) · oligo(dT) as template · primers. Enzymes were preincubated at 44° C and assayed at 34° C. The enzyme purified from mutant virions was inactivated about three times more rapidly at 44° C than that from RSV wild type virions (Fig. 1a and b). There was a small reproducible difference between the rates of inactivation of the two mutants, with the *ts337* enzyme being more heat sensitive.

To test the ability of the enzymes to transcribe RNA heteropolymers, we used 10S RNA from rabbit reticulocytes (globin mRNA) as a template with an oligomer of dT as primer⁴. Figure 1c shows that the rate of heat inactivation of the ability of the *ts337* enzyme to transcribe 10S RNA · oligo(dT) was identical to that measured with poly(C) · oligo(dG).

When heat-inactivation kinetics were assayed using a deoxyribohomopolymer, poly(dC) primed with oligo(dG), again enzymes from the mutants were inactivated more rapidly than the wild type enzyme. The enzymes from the mutants were inactivated slightly more slowly with this DNA template than with the homologous RNA template of poly(C), while the wild type enzyme was inactivated at the same rate with both templates. The *ts335* enzyme was again slightly less thermosensitive than the *ts337* enzyme. Using nuclease-treated calf thymus DNA¹¹, data indistinguishable from that in Fig. 2 were obtained with enzymes from both mutants and RSV wild type.

We investigated whether reduced binding of the enzyme from the mutants to the template might be partly responsible for their thermolability. Figure 3 shows that if template · primer was added to the reaction mixture during preincubation of DNA polymerases at 44° C, the wild type enzyme was protected against heat inactivation, whereas that of *ts335* was inactivated at the same rate as in the absence of template. These inactivation conditions, which show such a large difference between wild

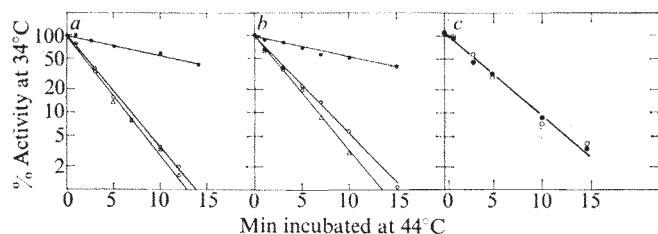


Fig. 1 Thermal inactivation of purified DNA polymerases from mutant and wild type virions using ribohomopolymers as templates. Purified virions of *ts335*, *ts337* and wild type RSV were obtained as described before². About 4 to 6 mg of viral protein was used for enzyme purification. Virions were lysed and the DNA polymerase purified by chromatography on DEAE-Sephadex and then on phosphocellulose columns as before⁶. DNA polymerase from the mutants *ts335* and *ts337* (clone B)² and wild type RSV (clone B)² eluted at a concentration of 0.06 to 0.08 M KCl from DEAE-Sephadex and 0.24 to 0.30 M KCl from phosphocellulose. A recovery of 40–50% of DNA polymerase was achieved by carrying out the procedures as rapidly as possible. Heat lability was assayed by preincubating the DNA polymerase in reaction mixture I, which contained 100 mM Tris-HCl, pH 8.3, 20 mM dithiothreitol, 12 mM magnesium acetate, 120 mM NaCl and 0.05–1.0 U of purified enzyme per 0.05 ml. (A unit of enzyme activity is defined as the amount of enzyme required to incorporate 100 pmol of ³H-dGMP at 37° C in 15 min⁶.) The reaction mixture was incubated at 44° C. At various intervals aliquots of 0.05 ml were withdrawn and added to 0.05 ml of reaction mixture II which contained template, primer and substrates. This was kept on ice for 10–15 min, then incubated at 34° C for 15 min. Acid-precipitable radioactivity was measured as described²⁴. The rate of the enzyme reaction at 34° C was linear for at least 15 min. a, Poly(C) (1.0 µg) and oligo(dG) (0.5 µg) were template-primer with 20 nmol ³H-dGTP (100 c.p.m. pmol⁻¹) as substrate. ●, RSV wild type; ○, *ts335*; △, *ts337*. b, Poly(A) (1.0 µg) and oligo(dT) (0.5 µg) were template-primer with 20 nmol ³H-dTTP as substrate. Symbols are as in (a). c, ●, rabbit reticulocyte 10S RNA (30 nmol nucleotide) and oligo(dT) (140 pmol nucleotide) were template-primer with 20 nmol ³H-dGTP (100 c.p.m. pmol⁻¹) and 60 nmol each of dATP, dCTP and dTTP as substrate and DNA polymerase from *ts337*. Reaction mixture II was incubated at 34° C for 60 min. ○, poly(C).oligo(dG) were template · primer as in (a).

type and mutant, may be more relevant to the *in vivo* situation than inactivation in the absence of template.

The purified DNA polymerases from *ts335*, *ts337* and wild type RSV all exhibit, in addition to DNA polymerase activity, a nuclease activity which selectively removes the RNA moiety from a DNA-RNA hybrid (ribonuclease H activity). Figure 4a shows the activity of polymerase-associated ribonuclease H from *ts335*, *ts337* and wild type RSV after incubation at 44°C for various times. The activity from the mutant was more sensitive to temperature than that from the wild type. However, the ribonuclease H activity was less sensitive to inactivation at 44°C than was the DNA polymerase activity¹². At 47°C the ribonuclease H activity of *ts337* DNA polymerase was much more thermolabile than the wild type enzyme (Fig. 4b).

The half lives of the synthetic and degradative activities of purified DNA polymerase from the mutants and the wild type RSV are collected in Table 2. The enzymes from the mutants were four to ten times more heat labile than those from the wild type virus.

Revertants and recombinants

Mason *et al.* (in preparation) isolated revertants of both *ts335* and *ts337* which are no longer temperature sensitive for the initiation of infection at 41°C. To see if the phenotypic reversion

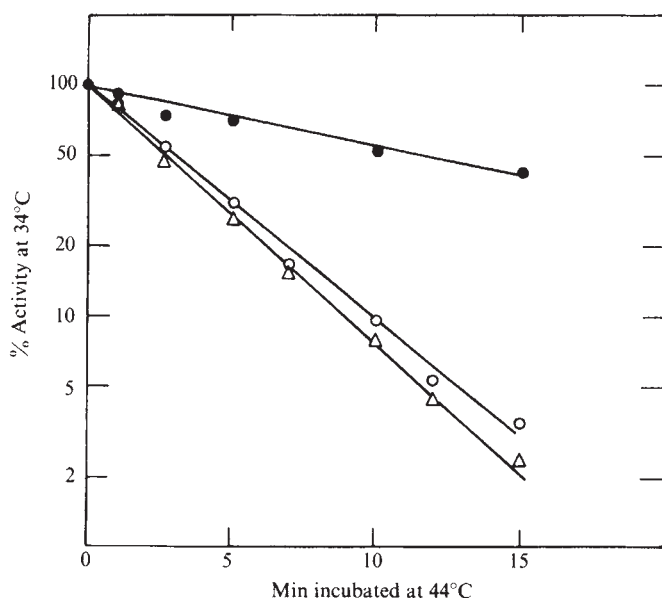


Fig. 2 Thermal inactivation of the DNA polymerase activity of mutant and wild type enzymes with poly(dC) · oligo(dG) as template. The same reaction conditions were used as described in the legend to Fig. 1a, except that 1.0 µg of poly(dC) was substituted for poly(C). ●, RSV wild type; ○, *ts335*; △, *ts337*.

was paralleled by a reversion of DNA polymerase to wild type behaviour, the heat inactivation kinetics of the DNA polymerase activity in virions of revertants of *ts335* and *ts337* were compared with that of mutant *ts337* utilising poly(C) · oligo(dG) and poly(dC) · oligo(dG) as template · primers. The virion-associated DNA polymerase activity of the revertants was three or four times more heat stable than that of the mutants (Fig. 5a and b). Similarly, the ribonuclease H activity of the DNA polymerase of the revertants was ten times more resistant to thermal inactivation than ribonuclease H activity from the mutants (Fig. 5c). Therefore both the biological and biochemical parameters vary together during selection for reversion of the temperature sensitive lesion.

Because the mutants were isolated from a transforming virus of host range subgroup C Prague RSV, recombinants could be selected from mixed infections with the group B non-transforming leukemia virus, RAV-6^{13,14}. Recombinants were selected as transforming viruses of group B. The temperature-

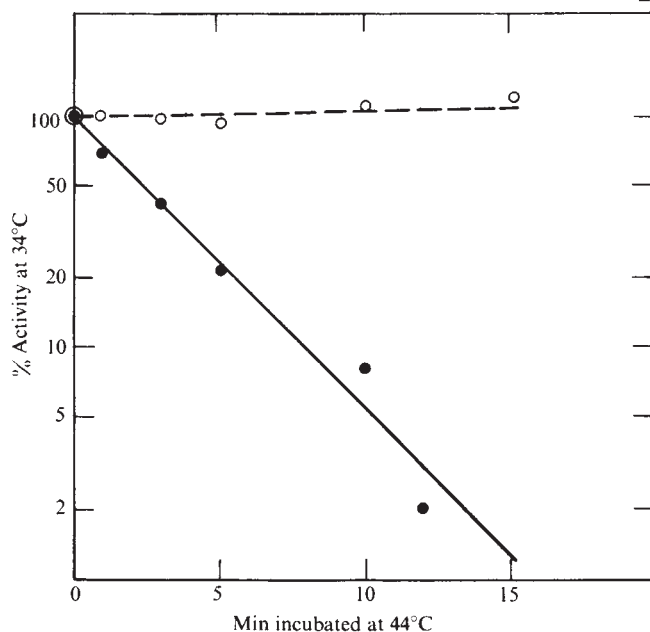


Fig. 3 Effect of template · primer on the rate of heat inactivation of mutant and wild type RSV DNA polymerase activity. The reaction was as described for Fig. 1a, except that template · primer was included in reaction mixture I. ○, RSV wild type; ●, *ts335*.

sensitive lesion was an unselected marker because permissive temperature was used throughout the selection. Recombinant clones were then screened for temperature sensitivity of growth and transformation (W. S. M., R. R. Friis, M. Linial and P. K. Vogt, in preparation).

Heat inactivation kinetics of the DNA polymerase activity in the virions of the wild type and temperature sensitive recombinants were assayed (Fig. 6). Both synthetic and degradative activities of the DNA polymerases of the wild type viruses were more thermostable than those of enzymes of the temperature sensitive recombinants.

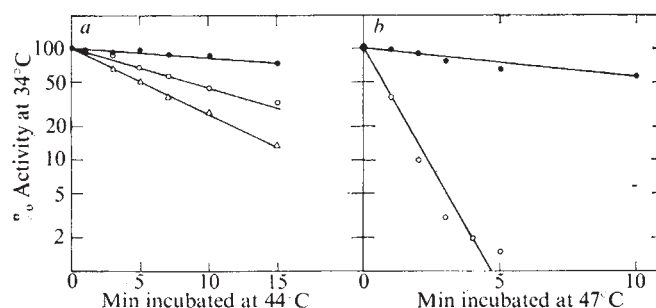


Fig. 4 Thermal inactivation of ribonuclease H activity of mutant and wild type RSV DNA polymerase. Ribonuclease H was assayed by using ³H-poly(A) · poly(dT) as substrate and monitoring the degradation of ³H-poly(A) to acid-soluble material. Heat inactivation was carried out as for Fig. 1 by preincubation at either 44°C or 47°C followed by incubation at 34°C. Reaction mixture I was as for Fig. 1a, except that 40 mM Mg acetate and 60 mM NaCl were used. Reaction mixture II contained 10 pmol of ³H-poly(A) (1,000 c.p.m. pmol⁻¹) and 2.8 pmol of poly(dT). The reaction at 34°C was carried out for 60 min under an atmosphere of nitrogen. The acid-soluble radioactivity was determined by first adding 10 µl of 10 mg ml⁻¹ bovine serum albumin and 30 µl of 50% trichloroacetic acid to the reaction mixture at the termination of incubation. After 15 min at 4°C, the samples were centrifuged in a bench-top centrifuge at full speed for 3 min and 100 µl aliquots were withdrawn from the supernatant and counted directly in 5.0 ml of dioxane-based scintillation fluid. The degradation of poly(A) by the unincubated enzyme is represented as 100%. All other values are normalised to the value of the unincubated sample. a, ●, RSV wild type; ○, *ts335*; △, *ts337*. b, ●, RSV wild type; ○, *ts337*.

Table 2 Average times for inactivation of half of the activities of the purified DNA polymerase from mutant and wild type RSV

Source	DNA polymerase activity at 44°		Ribonuclease H activity at	
	poly(C) · oligo(dG)	poly(dC) · oligo(dG)	44° C	47° C
RSV wild type	11.5	11.5	30	13
<i>ts335</i>	2	3	7.4	—
<i>ts337</i>	1.8	2.8	5	1.5

Values are expressed in min as $t_{1/2}$, which is the time required for inactivation of 50% the relevant activity as determined in Figs 1, 2 and 4.

Implications of thermolability

RNA tumour viruses contain a DNA polymerase which can utilise polyribonucleotides as templates to synthesise a faithful complementary DNA transcript⁴. This enzyme has been attributed a central role in the provirus hypothesis¹⁵. In the scheme of events postulated in the provirus hypothesis, such an enzyme needs to be operational just after infection for the synthesis of DNA complementary to viral RNA. However, it

All three activities of the purified DNA polymerase from avian tumour viruses show increased thermolability in the DNA polymerase of the mutant viruses, and therefore seem to reside in the same polypeptide chain. Grandgenett *et al.*⁸ have shown that the isolated, lower molecular weight subunit of AMV DNA polymerase manifests both the synthetic and the degradative activities of the whole enzyme. We have confirmed this for RSV and have isolated the small subunit of the DNA polymerase from *ts337* and wild type virus. Both synthetic and degradative activities of the isolated small subunit of *ts337* showed heat-inactivation kinetics indistinguishable from those of the complete *ts337* DNA polymerase (I.M.V., A. Panet and W.S.M., in preparation). The lower molecular weight subunit must therefore be encoded by viral RNA; the origin of the other subunit is under investigation.

We thank Dr John Wyke for isolating *ts335*, Dr G. F. Temple for globin mRNA, Drs Hung Fan and William Haseltine for stimulating discussions, Nora Meuth, Tad Nitasaka and Philip Harris for technical assistance and Dr Peter Vogt for generous support. This study was supported by contracts from the Virus-Cancer Program of the National Cancer Institute to D. B. and to Peter Vogt and by grants from the US Public

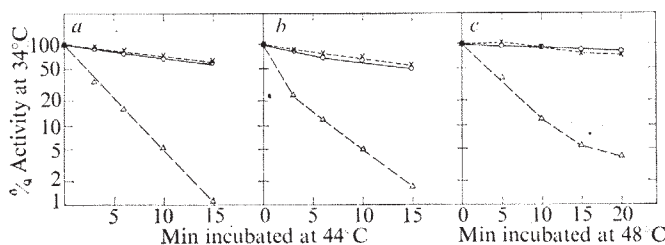


Fig. 5 Thermal inactivation of polymerase and nuclease activity associated with purified virions of revertants of *ts335* and *ts337*. Spontaneous revertants of *ts335* and *ts337* were selected by growth of the temperature sensitive viruses at the non-permissive temperature (41° C). Cloned revertant stocks were then isolated at the permissive temperature (35° C). For the experiments reported in this figure, 7.0 μ g of virion protein from the following stocks was used: *ts335* revertant clone A-3, *ts337* revertant clone γ -2 and *ts337* clone γ (W.S.M., R. R. Friis, M. Linial and P. K. Vogt, in preparation). *a*, Inactivation of polymerase activity measured with poly(C)·oligo(dG) as in Table 1. *b*, Inactivation of polymerase activity measured with poly(dC)·oligo(dG) as in Table 1. *c*, Inactivation of ribonuclease H activity measured as in Fig. 4. Preincubation was at 48° C. $\times$, *ts335* revertant; $\bigcirc$, *ts337* revertant; Δ , *ts337*.

has not previously been possible to determine unambiguously if the virion DNA polymerase is responsible for provirus synthesis and if the enzyme is coded by the viral genome or is cellular in origin. Virus-specificity of the enzyme has been suggested because it appears only after infection of the cell^{16,17}. The finding of noninfectious virions lacking DNA polymerase has been interpreted as evidence for an obligatory role for the enzyme¹⁸⁻²¹.

The study reported here demonstrates covariation of thermolability of the virion DNA polymerase with temperature sensitivity of infection and transformation by the virions. Two early temperature sensitive mutants of RSV were found to have DNA polymerase more thermolabile than that of the wild type virus, while revertants of the mutants (W. S. M., R. R. Friis, M. Linial and P. K. Vogt, in preparation) as well as a class of wild type recombinants² had DNA polymerase with wild type properties. This indicates that RSV RNA encodes the information for at least part of the DNA polymerase molecule and the enzyme must be necessary to initiate infection or transformation by RSV. Bishop *et al.*²⁷ reported that less viral DNA is produced by *ts335* and *ts337* at nonpermissive temperature than is produced by the wild type virus, which implies that the virus-encoded DNA polymerase is responsible for the synthesis of DNA after infection by RNA tumour viruses.

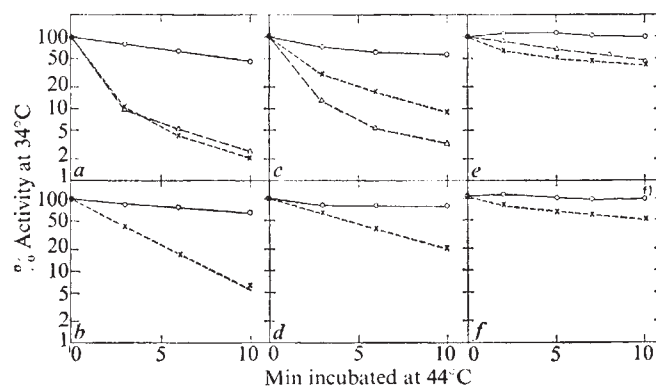


Fig. 6 Thermal inactivation of DNA polymerase and ribonuclease H activity of various stocks of recombinants. Recombinants between temperature sensitive RSV stocks of subgroup C and avian leukosis virus RAV-6 of subgroup B, were isolated as described (W.S.M., R. R. Friis, M. Linial and P. K. Vogt, in preparation)³. The recombinant sarcoma viruses had the subgroup B host range characteristic of RAV-6 and were of either wild type or temperature sensitive genotype. We have used the following nomenclature. *ts337* $\times$ RAV(WT) or *ts335* $\times$ RAV(WT): the mutant virus was co-cultivated with RAV-6 and the resultant viruses after appropriate screening could transform and replicate at non-permissive temperature (41° C). The following clones were used to prepare virus stocks: *ts337* $\times$ RAV(WT), clone 21; *ts335* $\times$ RAV(WT), clone 17 (in preparation as above). *ts337* $\times$ RAV(Ts) or *ts335* $\times$ RAV(Ts): the mutant virus was cocultivated with RAV-6 and the resultant viruses after appropriate screening could not transform or replicate at nonpermissive temperature (41° C). The following clones were used to make virus stocks: *ts337* $\times$ RAV(Ts), clone 7; *ts335* $\times$ RAV(Ts), clone 14 (in preparation as above). *a*, Poly(C) · oligo(dG) was template · primer. $\bigcirc$, *ts337* $\times$ RAV(WT); $\times$, *ts337* $\times$ RAV(Ts); Δ , *ts337*. *b*, Poly(C) · oligo(dG) was template · primer. $\bigcirc$, *ts335* $\times$ RAV(WT); $\times$, *ts335* $\times$ RAV(Ts). *c* and *d*, Poly(dC) · oligo(dG) was template · primer. Symbols as in *a* and *b*, respectively. *e* and *f*, Ribonuclease H assays. Symbols as in *a* and *b*, respectively.

Health Service and the American Cancer Society. I.M.V. was a fellow of the Jane Coffin Childs Memorial Fund for Medical Research; W.S.M. was a fellow of the Leukemia Society of America, Inc., D.B. was an American Cancer Society Professor of Microbiology.

Received March 25; revised April 24, 1974.

- ¹ Wyke, J. A., *Virology*, **52**, 587 (1973).
- ² Linial, M., and Mason, W. S., *Virology*, **53**, 258 (1973).
- ³ Vogt, P. K., Weiss, R. A., and Hanafusa, H., *J. Virol.*, **13**, 551 (1974).
- ⁴ Temin, H. M., and Baltimore, D., in *Advances in virus research*, **17**, 129 (Academic Press, New York, 1972).
- ⁵ Baltimore, D., and Smoler, D. F., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 1507 (1971).
- ⁶ Verma, I. M., and Baltimore, D., in *Methods in enzymology* (edit. by Grossman, L., and Moldave, K.), **29**, 125 (Academic Press, New York, 1973).
- ⁷ Verma, I. M., Meuth, N. L., Fan, H., and Baltimore, D., *J. Virol.*, **13**, 1075 (1974).
- ⁸ Grandgenett, D. P., Gerard, G. F., and Green, M., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 230 (1973).
- ⁹ Kacian, D. L., Watson, K. F., Burny, A., and Spiegelman, S., *Biochim. biophys. Acta*, **246**, 365 (1971).
- ¹⁰ Faras, A. J., Taylor, J. M., McDonald, J. P., Levinson, W. E., and Bishop, J. M., *Biochemistry*, **11**, 2334 (1972).

- ¹¹ Loeb, L. A., *J. biol. Chem.*, **244**, 1672 (1969).
- ¹² Leis, J., Berkower, I., and Hurwitz, J., in *DNA synthesis in vitro* (edit. by Wells, R. D., and Inman, R. B.), 287 (University Park Press, Baltimore, 1973).
- ¹³ Kawai, S., and Hanafusa, H., *Virology*, **49**, 37 (1972).
- ¹⁴ Vogt, P. K., *Virology*, **46**, 947 (1971).
- ¹⁵ *The molecular biology of tumour viruses* (edit. by Tooze, J.) Ch. 11 (Cold Spring Harbor Laboratory, Cold Spring Harbor, 1973).
- ¹⁶ Ross, J., Scolnick, E. M., Todaro, G., and Aaronson, S. A., *Nature new Biol.*, **231**, 163 (1972).
- ¹⁷ Baltimore, D., McCaffrey, R., and Smoler, D. F., in *Virus research* (edit. by Fox, C. F., and Robinson, W. S.), 51 (Academic Press, New York, 1973).
- ¹⁸ Hanafusa, H., Baltimore, D., Smoler, D. F., Watson, K. F., Yaniv, A., and Spiegelman, S., *Science*, **177**, 1188 (1972).
- ¹⁹ Peebles, P. T., Haapala, D. K., and Gazdar, A. F., *J. gen. Virol.*, **9**, 488 (1972).
- ²⁰ May, J. T., Somers, K. D., and Kit, S., *J. gen. Virol.*, **16**, 223 (1972).
- ²¹ Somers, K. D., May, J. T., Kit, S., McCormick, K. J., Hatch, G. G., Stenback, W. A., and Trentin, J. J., *Intervirology*, **1**, 11 (1973).
- ²² Bishop, J. M., Quintrell, N., Medeiros, E., and Varmus, H. E., *Cancer* (in the press).
- ²³ Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. biol. Chem.*, **193**, 265 (1951).
- ²⁴ Baltimore, D., Huang, A. S., and Stampfer, M., *Proc. natn. Acad. Sci. U.S.A.*, **66**, 572 (1970).

Membrane structure changes in rod outer segments associated with rhodopsin bleaching

P. A. Liebman, W. S. Jagger*, M. W. Kaplan & F. G. Bargoot

Department of Anatomy and Johnson Foundation, University of Pennsylvania, Philadelphia, Pennsylvania 19174

Static and time resolved birefringence analysis is used to quantitate a fast membrane structure change driven by the formation of metarhodopsin II in intact frog retina. This change can be accounted for by the disorientation of only one phospholipid molecule per rhodopsin bleached.

SCHMIDT'S discovery and analysis of the radially positive birefringence of myelin sheath and of the positive uniaxial birefringence of retinal rod outer segments (ROS) gave early evidence of a two-dimensional liquid crystalline structure for biological membranes¹. More recently, time-resolved birefringence measurements have been made in attempts to detect structural alterations, such as lipid reorientation, suspected to accompany action potentials in excitable membranes. Very small transient changes from the resting birefringence associated with action potentials of nerve, electroplax and muscle have been detected, usually by summing many responses in a signal averager²⁻⁶. Where sufficiently extensive analysis of these responses has been possible, however, the birefringence transients have been found to be secondary to the excitation process rather than part of its mechanism⁷. This does not mean that there is no primary alteration of membrane anisotropy, but rather that the surface density of sites at which such changes occur in these tissues may be too small to be detected, or may be small compared with the background of less interesting changes.

It is not surprising that little effort has been made to look for small changes in the birefringence of rods coincident

with excitation, since the light used to observe the birefringence may at the same time alter the system by bleaching its pigment: Nevertheless, our experience using infrared image converters and sensitive photomultipliers to deal with dark adapted ROS⁸, suggested that a birefringence analysis of this material might be feasible and would be especially worthwhile for the following reasons.

Individual frog ROS contain 2.5 mM rhodopsin in an 1,800 μm^3 volume⁹. Thus 3×10^9 equipotent trigger molecules¹⁰ are embedded in the 4,000 closely stacked lamellar membranes (2,000 disks), each of 28 μm^2 area to yield a surface density of 25,000 rhodopsins per μm^2 . This, for example, exceeds by 1,000 times the density of sodium channels in nerve¹¹. The 60- μm long ROS themselves lie parallel to one another in a dense array without intervening inactive material (cytoplasm, mitochondria, Schwann sheath, myelin) so predominant in nerve. In sum, the features of very high active membrane density and good alignment of extensive material of high purity provide a most appropriate subject for birefringence analysis.

ROS birefringence transient

Using an infrared dissecting microscope, dark adapted retinæ from *Rana pipiens* were isolated in frog Ringer solution. For static birefringence studies, outer segments were dislodged on a microscope slide by gently tapping a piece of retina against the slide. A drop of Ringer or 12% gelatin-Ringer solution was added, the preparation closed with a cover-slip and the edges blotted dry and sealed with paraffin wax as before¹². For kinetic studies, the retina was cut into a dozen or more strips, each of which curled spontaneously to form a tube with the ROS outermost. Each was transferred to a microscope slide and surrounded by a paper spacer to prevent squashing when the cover-slip and wax were added. These strips form so-called 'edge-fold'

*Present address: Max-Planck-Institut für Physiologische und Klinische Forschung, Bad Nauheim, West Germany.

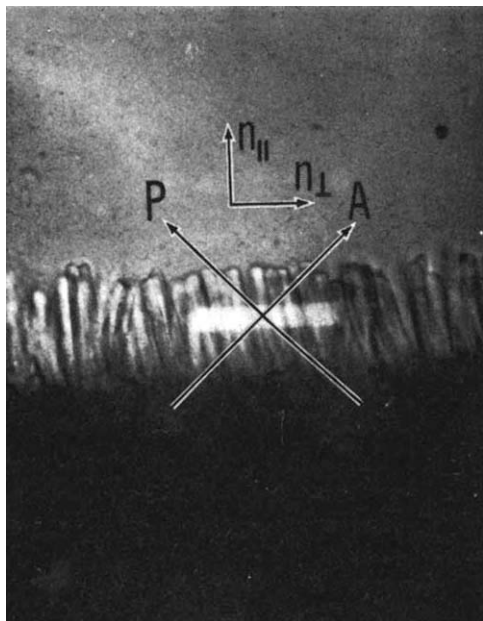


Fig. 1 Edge-folded retina showing aligned rows of rod outer segments with measuring beam superimposed. For birefringence measurement, light initially polarised at a 45° angle (P) with respect to the ROS axis traverses the cell. Since $n_{\parallel} > n_{\perp}$, the phase of the electric field component parallel to the rod axis is retarded with respect to the component perpendicular to it. An analyser (A) transmits only the minor axis component of the resultant elliptically polarised light emerging from the ROS. The birefringence, $\Delta n = n_{\parallel} - n_{\perp}$, is defined as axially positive when $n_{\parallel} > n_{\perp}$.

preparations in which aligned rows of ROS project from the edge of the retina¹³. Birefringence observations were made using a low numerical aperture polarising microscope equipped for infrared viewing and with an inverted objective lens as a condenser. A low intensity measuring beam of monochromatic light ($30 \mu\text{m} \times 300 \mu\text{m}$ at the specimen plane) was imaged on the ROS layer (Fig. 1). A 1-ms xenon bleaching flash beam was demagnified to the same dimensions as the measuring beam and superimposed on it using a beam splitter. The flash was made blue-green by a Wratten 47B filter together with a series of infrared absorbing glass filters. Its intensity was sufficient to bleach 50–60% of the pigment with each flash, a saturating bleach^{14,15}. The flash was blocked from the photomultiplier by a red-transmitting cutoff filter. Birefringence transients initiated by the flash were detected as a change in measuring beam intensity through a system of crossed polariser and analyser with the specimen in between. The axis of the ROS was at an angle of 45° with respect to the polariser axis (Fig. 1). Transmitted intensity was recorded using a storage oscilloscope directly coupled to the amplified output of the detecting photomultiplier (S-20 cathode).

To avoid problems of interpretation due to visual pigment absorption changes on bleaching, flash-induced birefringence changes were initially sought at wavelengths longer than 650 nm, outside the absorption band of rhodopsin. Exposure to a saturating flash initiated a rapid 6–8% loss of light transmission, corresponding to a 3–4% decrease in the net birefringence (Fig. 2). Flashes of reduced intensity caused proportionally smaller changes. The transmission loss had the action spectrum of rhodopsin. A $1/20\lambda$ retardation plate inserted between polariser and specimen served both to calibrate the instrument and to prove that the signal measured was a change in birefringence. When this plate was positioned so as to add to the specimen birefringence, the flash-induced signal was a reduction of transmitted light intensity. When the plate was rotated to produce a total static retardation of the same magnitude but of opposite

sign, the signal was inverted. This shows that the flash-induced signal was not due to absorption, optical rotation or light scattering changes in the specimen, but that it was due to a change in birefringence.

Wavelength dependence

In experiments using measuring lights of different wavelength, the birefringence transient (BRT) was found to be achromatic at wavelengths between 700 nm and 650 nm, to vanish between 650 nm and 600 nm and to be reversed in sign (birefringence increase) from 600 nm to 550 nm, increasing in magnitude with decreasing wavelength. This peculiar wavelength-dependent behaviour of the BRT was understood through measurement of the static wavelength dependence of the birefringence of single dark adapted ROS. Our dual beam low-light-level wavelength scanning microspectrophotometer⁸ was altered by insertion of crossed polarisers and a $1/20\lambda$ retardation plate to allow birefringence recording. The logarithm of the transmission ratio between a region containing the retardation plate alone and that containing a single ROS plus retardation plate was measured using microbeams of cellular dimensions without detectable visual pigment bleaching. Figure 3a shows the results of such a recording with the slow axis of the retardation plate parallel to the ROS long axis (the birefringence slow axis). Reversal of the retardation plate orientation leads to mirror inversion of the recorded spectrum, indicating that the entire spectrum is due to birefringence. The effect of fractional bleaches of the visual pigment is immediately to reduce the peak-to-peak amplitude of the spectral excursions. A slow achromatic increase in birefringence also occurs subsequent to bleaching with a half-time of about 20 min at 20°C . The retardation spectrum in Fig. 3b was computed from the recorded transmission spectrum using the formulae derived by Szivessy¹⁶. The resulting curve can be understood in the following way. When matter interacts

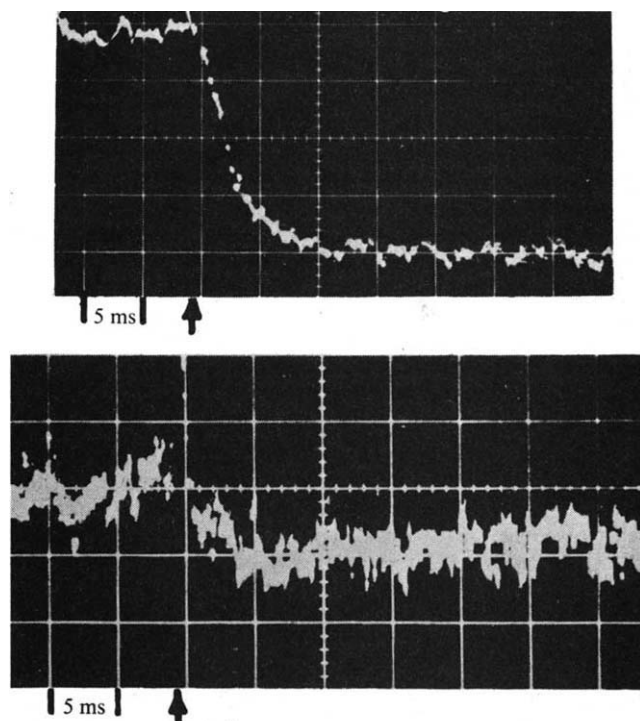


Fig. 2 Comparison of metarhodopsin II (MII) formation and the BRT. The upper oscilloscope trace shows the *in situ* loss of light transmission at $\lambda=380 \text{ nm}$ due to a flash induced (at arrow) increase in MII concentration. The temperature was 21.5°C . The lower trace shows the BRT at $\lambda=680 \text{ nm}$ under the same conditions. The temperature was 21.8°C .

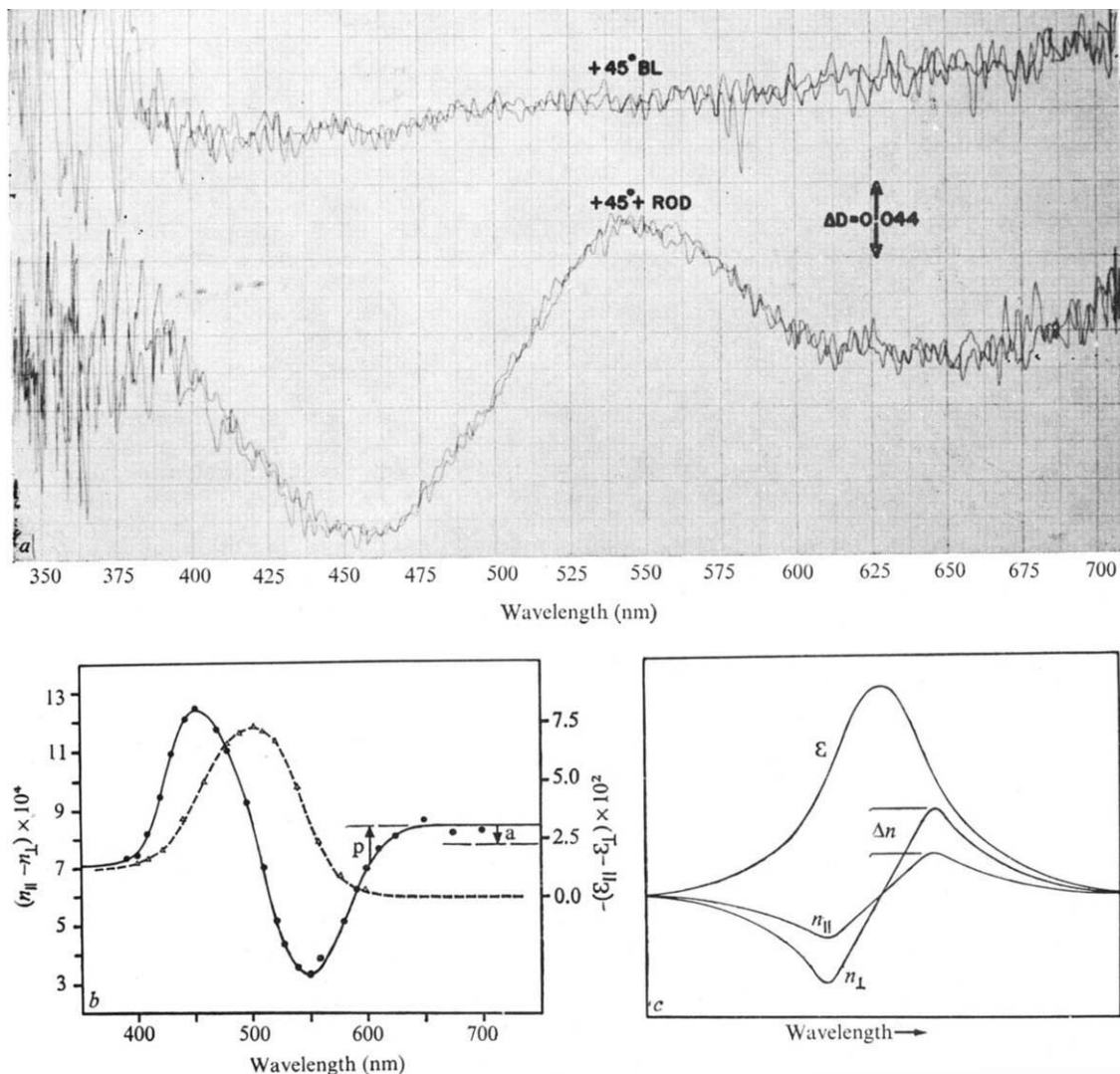


Fig. 3 *a*, Wavelength dependence of transmitted light intensity due to dark adapted ROS static birefringence plus a $\lambda/20$ plate aligned with its slow axis along the ROS axis, 45° from the polariser axis ($+45^\circ + \text{ROD}$). Superposition of two successive scans shows that no bleaching occurs during the measurement. Baseline taken in the absence of the ROS ($+45^\circ \text{ BL}$). Intensity increases downward. ΔD is the equivalent optical density change. *b*, Birefringence spectrum (●) computed from data of (*a*). Arrow *a* indicates the flash-induced achromatic birefringence loss. Arrow *p* indicates the wavelength-dependent birefringence change due to loss of visual pigment related anomalous dispersion differences. The direction and magnitude of the BRT depends on the relative magnitudes of *a* and *p* at a given

wavelength. Dichroism spectrum (Δ) showing a higher absorption coefficient for the perpendicular (to the rod axis) component than for the parallel component, the maximum difference occurring at the absorption peak of rhodopsin. *c*, Schematic diagram showing that the anomalous dispersion of the refractive index in the region of the rhodopsin absorption peak is more pronounced for the perpendicular direction, $n_{\perp}$, than for the parallel direction, $n_{\parallel}$, due to dichroism of the visual pigment. For wavelengths longer than the absorption peak, the anomalous dispersion-dependent birefringence component is negative ($n_{\parallel} - n_{\perp} < 0$). Loss of this negative component on bleaching contributes an increase to the net birefringence.

with light, in the region of an absorption band, there can be both absorption of photons and a phase change (retardation) of the transmitted wave. These properties are expressed as the imaginary and the real part of the complex index of refraction respectively. The two components of the complex index are mathematically related by the Kramers-Kronig or other dispersion relationships^{17,18}. In spectral regions distant from the absorption peak, the real part of the refractive index, n , decreases with increasing wavelength ($dn/d\lambda < 0$). However, in the region of the absorption peak, n increases with increasing wavelength ($dn/d\lambda > 0$), producing an anomalous dispersion effect. Intact ROS are dichroic because of the net orientation of rhodopsin chromophores in the membrane. Absorption of light polarised perpendicular to the rod axis (in the plane of the lamellar membranes) is four to five times more efficient than of light polarised parallel to the rod axis¹². Similarly, this means that the anomalous dispersion associated with rhodopsin absorption will be more pronounced

for the perpendicular component of the refractive index, $n_{\perp}$, than for the parallel component, $n_{\parallel}$, as shown schematically in Fig. 3c. Our birefringence spectrum in the region of the absorption peak measures the difference, $\Delta n = n_{\parallel} - n_{\perp}$, between the dispersion curves for the two polarisations. Note that this anomalous dispersion component of the overall birefringence is negative with respect to the rod axis for wavelengths longer than the absorption peak.

Possible sources of BRT

Could the disappearance of the visual pigment anomalous dispersion effect as a result of bleaching then explain the observed long-wavelength BRT. Evidently not, for this loss of negative birefringence would cause an increase in the (positive) net birefringence whereas a decrease is actually observed. If the long-wavelength BRT, however, is due to the concurrent loss of a wavelength-independent positive contribution to the net birefringence, such as would

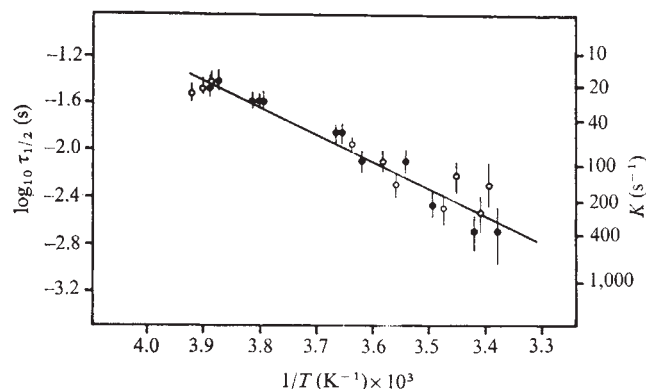


Fig. 4 Arrhenius plots of the half-times of MII formation (○) and of the BRT (●) for an edge-folded retina. The equivalent first order rate constant scale is on the right.

be associated with a lipid phase transition of the disk membranes, or to the gain of a negative one, we would expect to find some shorter wavelength at which the gain in birefringence due to loss of the anomalous dispersion effect would be just offset by the loss in birefringence caused by the achromatic component. This is in fact the case, as described above.

Although this analysis shows that the achromatic BRT cannot be attributed to a loss of the visual pigment anomalous dispersion effect, its millisecond relaxation time suggested that it is linked to the formation of the photo-product metarhodopsin II (MII), which appears at a similar rate in solution²¹. To test this hypothesis we measured the half-times of *in situ* MII formation and of the BRT (Fig. 2) as a function of temperature, using edge-folded retina preparations mounted on a thermostatically controlled Peltier cooler on the microscope stage. Coincidence of the Arrhenius plots of these measurements is taken to support the notion that the BRT is closely linked to the formation of MII (Fig. 4). Formation of MII might only be a rate-limiting trigger, however, and the nature of its linkage to the BRT remained to be explored.

In studies on bleached ROS, Schmidt showed that the total static birefringence is the sum of two opposing components, the form and the intrinsic birefringence, $n_T = n_F + n_I$. A body constructed of layers of membrane alternating with cytoplasm must have a negative form birefringence, independent of considerations of molecular orientation, that is quantitatively predicted by Wiener's equation²⁰:

$$(n_{||} - n_{\perp})_F \equiv \Delta n_F = - [f_c f_m (n_m^2 - n_c^2)^2] / [2n_T (f_c n_m^2 + f_m n_c^2)] \quad (1)$$

where Δn_F is the form birefringence, f is volume fraction, n is refractive index and the subscripts m, c, and T refer to membrane, cytoplasm and mean index for the whole body, respectively. Schmidt reasoned that since ROS show net positive birefringence ($n_{||} > n_{\perp}$), there must also be an intrinsic component that is positive and of greater magnitude than the negative form component. He concluded that the intrinsic component resulted from net orientation of the lipid hydrocarbon chains perpendicular to the plane of the membrane, giving a higher refractive index along the chains than perpendicular to them. This constituted the discovery of membrane 'crystallinity'.

From the Wiener equation it is easy to see that a small change in the cytoplasmic volume fraction, f_c , could cause Δn_F to change. This might be mediated by an MII-driven change in osmotic pressure of the cytoplasm secondary to ion flow or a chemical dissociation reaction. It has also been shown, using X-ray diffraction techniques, that at some time during the 4–8 h after bleaching, rhodopsin has

sunk from a position of $\frac{1}{2}$ immersion to a position of $\frac{1}{3}$ immersion in the frog lamellar membranes²¹. Since the membrane refractive index, n_m , is due to both lipid and embedded protein, and the protein refractive index is higher than that of lipid, increased protein immersion on bleaching would cause n_m to increase at the expense of n_c . Wiener's equation predicts that such a redistribution of refractive index components would cause an increase in the magnitude of the negative form birefringence, again consistent with our measured result if this change were driven by the formation of MII. Alternatively, a change in disk membrane surface potential concurrent with MII formation such as could be associated with an ionic redistribution or the early receptor potential, might cause compression and/or expansion of the membrane or a Kerr effect dipole realignment, as has been postulated for nerve by Cohen *et al.*^{2,6,7}. This could change n_m , f_m or both.

To determine whether ionic mechanisms might be involved in either osmotic or surface potential effects, we looked for the BRT in retinæ immersed in Ca^{2+} -free, Na^{+} -free, K^{+} -free and Cl^{-} -free media and in media containing tetrodotoxin or ouabain. The BRT during all these treatments was indistinguishable from normal. In addition, the BRT persisted in frozen ($< -10^\circ \text{C}$) and in glutaraldehyde-fixed retinæ. Clearly, the BRT is not dependent on shifts in water distribution or on ionic diffusion potentials, known to be important in nerve. We therefore ruled these out as causal agents.

To examine the possibility that the BRT is caused by rhodopsin sinking into the lamellar membrane concurrent with MII formation, we note the finding of Korenbrot *et al.*²² that glycerol readily enters ROS and permeates the cytoplasmic space. Using the glycerol imbibition method, we did index matching experiments, analogous to those done by Blasie using X-rays, with the advantage of the millisecond time resolution of the birefringence measurement. Retinæ or single ROS were equilibrated in a series of Ringer solutions containing increasing fractions of glycerol. Since the refractive index of glycerol is 1.47, the

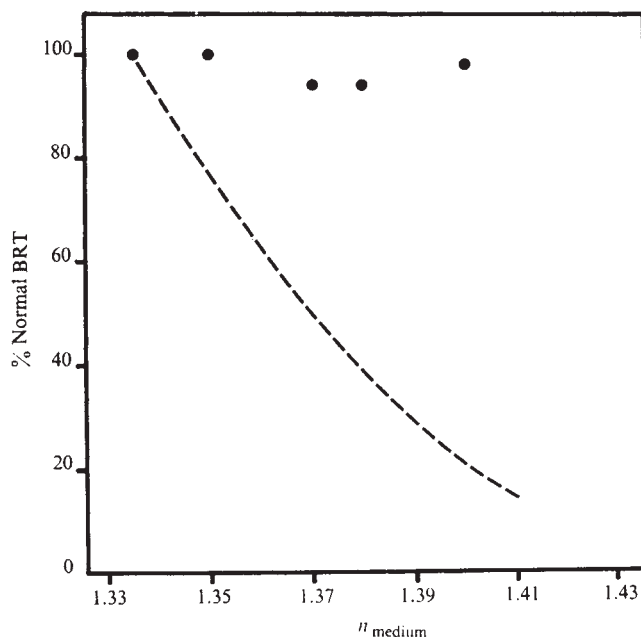


Fig. 5 Effect of glycerol imbibition on the relative magnitude of the BRT. Static form birefringence measurements indicate that, if the BRT was due to a change in form birefringence, the experimental points should lie on the dashed line. Lack of dependence of the BRT on imbibing medium refractive index, n_{medium} , shows that the achromatic BRT is due to a loss of intrinsic birefringence.

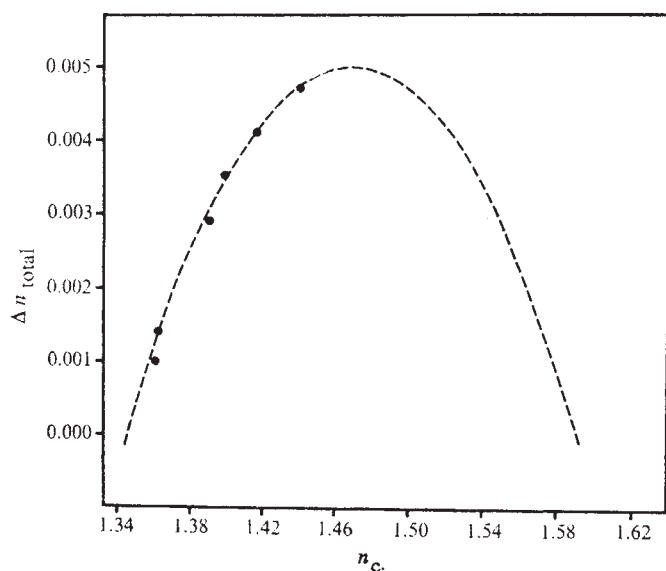


Fig. 6 Static birefringence imbibition curve of single ROS. Experimental points (●) lie on a plot of the Wiener equation for which the following best fit values have been used: $n_m=1.475$, $n_c=1.365$, $f_m=0.37$ and $f_c=0.63$. The curve shows that the net static birefringence of $+0.0010$ in Ringer solution is the sum of $+0.0050$ intrinsic component and a -0.0040 form component.

effect of these treatments was progressively to diminish refractive index contrast between membrane and cytoplasm as the cytoplasm index increased. If the BRT is caused by refractive index redistribution due to sinking of part of the rhodopsin molecule from cytoplasm to membrane, the influence of this redistribution should be diminished as the cytoplasmic index increases, up to the point where the index of the cytoplasm (glycerol) matches that of the non-rhodopsin part of the membrane. At match, a sinking rhodopsin can only move between two media of identical refractive index and its effect on the birefringence should then be eliminated. The results of these experiments show that the BRT is independent of the cytoplasmic refractive index and thus of the magnitude of the form birefringence component (Fig. 5). This rules out the sinking of rhodopsin as a cause of the BRT. More importantly it shows that the bleach-induced sinking of visual protein inferred by Blasie cannot occur on a time scale concurrent with visual excitation. The hypothesis that visual protein might float on one surface of the membrane in darkness and then, on light excitation, sink to bridge the lipid phase with a pore, allowing Ca^{2+} to escape, is therefore not supported by our measurements.

The experiments described here provide strong evidence that the achromatic BRT signals either lipid reorientation or a change in protein conformation, or both. A small negative activation entropy calculated from our Arrhenius plots of MII formation argues against a sizeable membrane conformation change, although it is difficult to say how much would be enough. It is also conceivable that compensating entropic changes occur in different parts of the lipid-protein complex, thus masking the true size of a conformational transition. One of these changes might be optically anisotropic while the other is not.

Birefringence and rod structure

We were able to learn something further of rod structure by measuring their static birefringence in glycerol-containing media of increasing refractive index. The result (Fig. 6) constitutes a so-called imbibition curve whose form is given by the Wiener equation. As described above, glycerol imbibition allows the form birefringence to

be eliminated progressively. It not only unmasks the positive intrinsic birefringence previously compensated by the nearly identical negative form birefringence, but it allows the values of n_o , n_m , f_c and f_m to be determined. From these measurements we find Δn_F to be -0.0040 for dark adapted *Rana pipiens* rods. Since we measure the total birefringence of normal dark adapted cells to be $+0.0010$, the intrinsic birefringence, Δn_I , is $+0.0050$. Other values obtained by fitting the curve to Wiener's equation are $n_m=1.475$, $n_o=1.365$, $f_m=0.37$ and $f_c=0.63$. Since the refractive index for Ringer solution is 1.336, the value $n_o=1.365$ suggests that the cytoplasmic space contains about 16% solids, based on the mean refractive increment for sugars and proteins of ~ 0.016 per g % (ref. 23). More than half of this value can be accounted for by protrusion of rhodopsin molecules from the membrane surface into the cytoplasmic space, while the remainder might be due to the protruding saccharide groups of glycolipids. Cytoplasmic bridges between disk membranes have been reported²⁴, and similar structures have been postulated to account for the restorative force in osmotic shrinkage experiments on ROS²⁵. The cytoplasmic volume fraction, f_o , is in reasonable agreement with estimates of other workers^{22,26}.

It is useful to compare our value for the intrinsic birefringence of ROS, $+0.0050$, with that of myelin, $+0.011$ (ref. 27). Although the two-fold difference suggests a more crystalline lipid order in myelin, it must be remembered that the large cytoplasmic volume fraction of ROS dilutes the measured membrane birefringence by more than a factor of two²⁸. Thus the true membrane value for ROS is about the same as for myelin. In view of the higher degree of unsaturation of ROS membrane phospholipids^{29,30} and low cholesterol content³¹, this result is rather surprising and implies that ROS protein may impose a more crystalline order on the ROS lipids than would occur in an equivalent protein-free bilayer. Alternatively, rhodopsin may itself be birefringent.

Implications

If the BRT is due to a loss in crystallinity of the lipids of ROS membranes, it is interesting to learn what fraction of the total crystallinity it represents. The ratio of the BRT to the intrinsic birefringence is about 1% when extrapolated to complete rhodopsin bleaching. Since there are about 100 phospholipid molecules per rhodopsin in ROS disk membranes³², the BRT could, therefore, be due to the disorientation of only one phospholipid molecule per rhodopsin bleached. Alternatively, a small anisotropic change in protein structure as MII is formed might account for our results.

On the basis of changes in ultraviolet absorption^{33,34}, ultraviolet circular dichroism^{35,36}, chemical reactivity^{37,38} and molecular size³⁹ of detergent-solubilised rhodopsin, previous workers have concluded that a change in protein conformation accompanies bleaching. Nevertheless, circular dichroism measurements on rod suspensions fail to confirm this evidence when rhodopsin is in its native milieu^{40,41}. Though flash photolysis measurements have been said to point independently to a conformation change during MII formation because of a large positive activation entropy found with this step^{42,43} such measurements have been confined largely to detergent-solubilised rhodopsin or to suspensions of previously frozen or otherwise aged rod particles. Contemporary evidence suggests that both these conditions alter the activation parameters found in fresh material⁴⁴ and we in fact find a negative activation entropy in our work on fresh rods. Thus, it is difficult to judge the significance of either the very specific assays of conformation change such as circular dichroism or of the less specific activation energy analyses for our work. In seeking an explanation for the BRT in intact

retinae, however, it seems that a change in protein conformation must occur, for without such a prime mover, there would be no evident trigger for a lipid reorientation. On the other hand, if the BRT does not signal lipid reorientation, it must be directly detecting a protein conformation change.

There is keen interest in the use of biophysical techniques to detect and identify dynamic properties associated with physiological function of cell membranes or their isolated constituents. The ideal detection technique would be sensitive, time resolved, and specific for a particular molecule or molecular property. Measurements would be possible on intact cells without significant perturbations in structure through introduction of extrinsic probes. Needless to say, the ideal does not exist in a single technique and choice is frequently dictated by problems peculiar to each tissue.

Though it is easy to fault birefringence analysis on the basis of poor specificity, we have found in its application to rod outer segments a technique that is sensitive, time resolved, without perturbation and applicable to intact cells. In addition, specificity may be designed into the experiment. It has thus been possible for us to detect a membrane structural alteration in intact visual receptors that is specifically linked to one stage in pigment bleaching and that is fast enough to be associated with visual excitation.

This work was supported by a grant and fellowships from the National Eye Institute. Parts were done in partial fulfilment of the requirements for the PhD degree (W. S. J., thesis, University of Pennsylvania, Philadelphia).

Received April 25, 1974.

- ¹ Schmidt, W. J., *Kolloidzeitschrift*, **85**, 137 (1938).
- ² Cohen, L. B., Keynes, R. D., and Hille, B., *Nature*, **218**, 438 (1968).
- ³ Tasaki, I., Watanabe, A., Sandlin, R., and Carnay, L., *Proc. natn. Acad. Sci. U.S.A.*, **61**, 883 (1968).
- ⁴ Cohen, L. B., Hille, B., and Keynes, R. D., *J. Physiol.*, **203**, 489 (1969).
- ⁵ Carnay, L. D., and Barry, W. H., *Science*, **165**, 608 (1969).
- ⁶ Cohen, L. B., Hille, B., and Keynes, R. D., *J. Physiol.*, **211**, 495 (1970).
- ⁷ Cohen, L. B., Keynes, R. D., Landowne, D., and Rohas, E., *J. Physiol.*, **218**, 205 (1971).
- ⁸ Liebman, P. A., in *Handbook of sensory physiology*, VII/1 (edit. by Dartnall, H. J. A.) (Springer, New York, 1972).
- ⁹ Liebman, P. A., *Biophys. J.*, **2**, 161 (1962).

- ¹⁰ Pirenne, M. H., in *The eye*, **2** (edit. by Davson, H.), Ch. 4 (Academic Press, New York, 1962).
- ¹¹ Hille, B., *Prog. Biophys. molec. Biol.*, **21** (edit. by Butler, J. A. V., and Noble, D.) (Pergamon, New York, 1970).
- ¹² Liebman, P. A., and Entine, G., *Vision Res.*, **8**, 761 (1968).
- ¹³ Denton, E. J., *J. Physiol.*, **124**, 16P (1954).
- ¹⁴ Hagins, W. A., *J. Physiol.*, **129**, 22 (1955).
- ¹⁵ Williams, T. P., *Vision Res.*, **10**, 789 (1970).
- ¹⁶ Szivessy, G., in *Handbuch der physik*, **20** (edit. by Geiger, H., and Scheel, K.), Ch. 11 (Springer, Berlin, 1928).
- ¹⁷ Kramers, M. H. A., *Atti Congr. int. Fisici*, Como, **2**, 545 (1927).
- ¹⁸ Davidov, A. S., *Izvestia Akademii Nauk SSSR Seria Fizicheskaya*, **17**, 523 (1953).
- ¹⁹ Williams, T. P., and Breil, S. J., *Vision Res.*, **8**, 777 (1968).
- ²⁰ Wiener, O., *Abh. sachs. Ges. Wiss., Leipzig*, **33**, 507 (1912).
- ²¹ Blasie, J. K., *Biophys. J.*, **12**, 191 (1972).
- ²² Korenbrot, J. I., Brown, D. T., and Cone, R. A., *J. Cell Biol.*, **56**, 389 (1973).
- ²³ Barer, R., in *Physical techniques in biological research*, IIIA (edit. by Pollister, A. W.), Ch. 1 (Academic Press, New York, 1966).
- ²⁴ Godfrey, A. J., *J. ultrastr. Res.*, **43**, 228 (1973).
- ²⁵ Korenbrot, J. I., and Cone, R. A., *J. gen. Physiol.*, **60**, 20 (1972).
- ²⁶ Weale, R. A., *Pflugers Arch.*, **329**, 244 (1971).
- ²⁷ Schmitt, F. O., and Bear, R. S., *J. cell. comp. Physiol.*, **9**, 261 (1937).
- ²⁸ Worthington, C. R., *A. Rev. Biophys. Bioengng* (in the press, 1974).
- ²⁹ Mason, W. T., Fager, R. S., and Abrahamson, E. W., *Biochemistry*, **12**, 2147 (1973).
- ³⁰ Anderson, R. E., and Risk, M., *Vision Res.*, **14**, 129 (1974).
- ³¹ Eichberg, J., and Hess, H. H., *Experientia*, **23**, 993 (1967).
- ³² Zorn, M., and Futterman, S., *J. biol. Chem.*, **246**, 881 (1971).
- ³³ Hubbard, R., Bownds, D., and Yoshizawa, T., *Cold Spring Harbor Symp. quant. Biol.*, **30**, 310 (1965).
- ³⁴ Ebrey, T. G., and Honig, B., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 1897 (1972).
- ³⁵ Crescitelli, F., Mommaerts, W. F. H. M., and Shaw, T. I., *Proc. natn. Acad. Sci. U.S.A.*, **56**, 1729 (1966).
- ³⁶ Takezaki, M., and Kito, Y., *Nature*, **215**, 1197 (1967).
- ³⁷ Falk, G., and Fatt, P., *J. Physiol.*, **183**, 211 (1966).
- ³⁸ Bownds, D., and Wald, G., *Nature*, **205**, 254 (1965).
- ³⁹ Heller, J., *Biochemistry*, **7**, 2914 (1968).
- ⁴⁰ Shichi, H., Lewis, M. S., Irreverre, F., and Stone, A. L., *J. biol. Chem.*, **244**, 529 (1969).
- ⁴¹ Cassim, J. Y., Rafferty, C. N., and McConnell, D. G., *Biophys. J.*, **12**, 205a (1972).
- ⁴² Pratt, D. C., Livingston, R., and Grellman, K. H., *Photochem. Photobiol.*, **3**, 121 (1964).
- ⁴³ Matthews, R. G., Hubbard, R., Brown, P. K., and Wald, G., *J. gen. Physiol.*, **47**, 215 (1963).
- ⁴⁴ Ebrey, T. G., *Vision Res.*, **8**, 965 (1968).
- ⁴⁵ Hagin, W. A., *Nature*, **177**, 989 (1965).
- ⁴⁶ Bargoot, F., thesis, Florida State Univ., Tallahassee (1972).

letters to nature

Large outbursts in Cygnus X-3

Two radio outbursts in Cyg X-3 were observed during December 1973 and January 1974. The source was observed at 4.2 GHz using the 26-m radio telescope of Kashima Branch of the Radio Research Laboratories, which has a half power beamwidth of 10 arc min. Flux densities were obtained using drift scan so as to avoid interference from HII regions surrounding Cyg X-3. An average of four or five scans was taken to determine the flux densities. The receiver gain was calibrated during each determination using the output from a noise tube. It was also calibrated

at five hour intervals by observing 3C147 (assumed flux density 9.3 Jy—1 Jansky=1 f.u.—) and 3C274 (80 Jy) in December 1973, and 3C123 (19.0 Jy) in January 1974. We measured the total flux densities alternately in orthogonal planes of polarisation by rotating the polariser, except on December 23 and 24, 1973. On those occasions the flux densities were measured in only one plane of polarisation. The pointing accuracy was checked frequently by the scans with declination offset. The error in flux density is less than $0.3 + 0.05 S_{4.2}$ Jy, where $S_{4.2}$ is the flux density at 4.2 GHz.

An outburst was first recognised at 2200 UT on December 23, 1973, with a flux density of about 6.5 Jy. On December

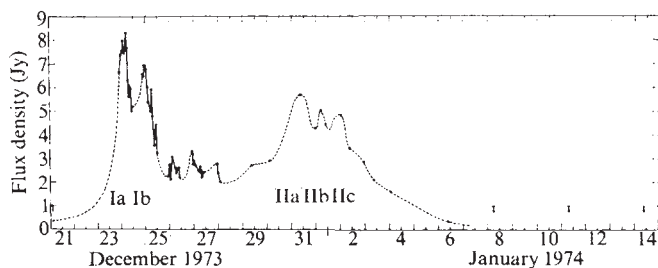


Fig. 1 Observed variation in the flux density of Cyg X-3 at 4.2 GHz. Peaks are numbered Ia–IIc.

21 the flux density of the source had been less than the noise level (upper limit 1 Jy). Five hours after it was first detected, the flux density reached its maximum of 8.30 Jy (Fig. 1). Seven days after the first event, a second outburst was observed. The second outburst was observed only a few times a day.

The two outbursts occurred about a week apart and both had a few peaks, each lasting about a day (Fig. 1). Both of these features are similar to those observed during the outbursts of Cyg X-3 in September 1972 (refs 1–3)—before now the only large event known to have occurred on the source since its radio identification on July 19, 1971 (ref. 4). It has, however, been monitored at intervals of a few months at Kashima since January 31, 1973, and except for this event, the flux densities have not exceeded 1 Jy.

We thank F. Takahashi and T. Miki for their collaboration. This work was one of the cooperative observations

between Radio Research Laboratories, Japan, and the Tokyo Astronomical Observatory, University of Tokyo.

TSUNEAKI DAISHIDO

Department of Astronomy,
University of Tokyo,
Tokyo, Japan

NOBUYUKI KAWANO
NOBUHIRO KAWAJIRI

Kashima Branch,
The Radio Research Laboratories,
Kashima, Ibaraki, Japan

MAKOTO INOUE

Department of Physics,
Nagoya University,
Nagoya, Japan

MASAHIRO KONNO

Department of Astronomy,
University of Kyoto,
Kyoto, Japan

Received March 21; revised April 8, 1974.

¹ Gregory, P. C., Kronberg, P. P., Seaquist, E. R., Hughes, V. A., Woodsworth, A., Viner, M. R., and Retallack, D., *Nature*, **239**, 440–443 (1972).

² Hjellming, R. M., and Balick, B., *Nature*, **239**, 443–446 (1972).

³ Braes, L. L. E., Miley, G. K., Shane, W. W., Baars, J. W. M., and Goss, W. M., *Nature phys. Sci.*, **242**, 66–69 (1973).

⁴ Braes, L. L. E., and Miley, G. K., *Nature*, **237**, 506–508 (1972).

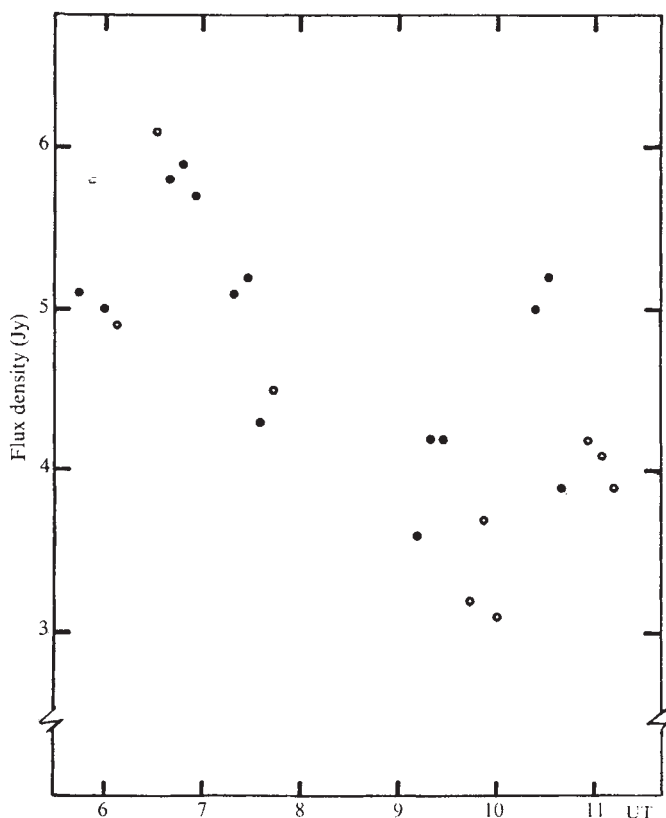


Fig. 2 Fine structure of the intensity variation on December 25, 1973, showing the spikes on peak Ib at 0644 and 1100 UT, respectively. Each point was obtained from one drift scan. Filled circles, observations at 0° (vertical) of the telescope polariser angle; open circles, 90° (horizontal) of the telescope polariser.

Timing results for thirteen pulsars

As part of a continuing programme of pulsar timing measurements at the Arecibo Observatory, timing results have been obtained for thirteen pulsars following a year or more of observations at 430 MHz. Precision timing results for these thirteen objects have not been reported previously. The discovery names, references, and relevant least-squares fitting information are given in Table 1. The fitted parameters right ascension, declination, period (P) and period derivative (dP/dt) are listed in Table 2.

The techniques used in the observations were basically the same as in previous work at Arecibo^{1,2}. Arrival times were determined by the template-matching method²; the template in the present case being the sum of a large number of pulses processed by an incoherent de-dispersing device³. The error bars assigned to daily average arrival times are the standard deviation from the mean of all the arrival times (usually ten or more) within the day. Ephemeris corrections were carried out using data supplied by the Massachusetts Institute of Technology Lincoln Laboratory and were based on the work of Ash, Shapiro and Smith⁴. The errors assigned to the fitted parameters in Table 2 are twice the formal standard errors resulting from the least-squares fitting process, the latter being computed according to Jurgens (unpublished) in order to reflect explicitly the value of χ^2 .

There are several aspects of the results which require comment. Fits for several of the pulsars yielded a χ^2 rather small compared with the number of degrees of freedom. The errors in the daily-average arrival times are apparently overestimated in these cases, perhaps as a result of 'pulse jitter' predominantly on time scales of several minutes. The two pulsars PSR0611+23 and PP0943 behave in the opposite way, having a much larger value of χ^2 than expected. It may well be that these two objects are further examples of pulsars exhibiting erratic behaviour in pulse phase on time scales greater than a few minutes, as do the Crab pulsar⁵, PSR1642–03 and PSR0329+54 (ref. 17). Another possible explanation is in terms of the changes in pulse shape that are seen in averages of several minutes for both

Table 1 Information on pulsars for which timing results were obtained by least squares

Serial no.	Discovery name	Ref.	Interval fitted (day/month/year)	Degrees of freedom	χ^2	R.M.S. Residual (μ s)	$\tau = P/(dP/dt)$ (10^9 yr)
1	PSR0301+19	6, 7	14/08/72-06/04/74	16	7.2	766	34
2	PSR0540+23	8, 9	26/01/73-07/03/74	11	2.0	146	0.51
3	PSR0611+22	8, 9	26/01/73-08/03/74	12	43.3	643	0.18
4	PP0943	10, 11	14/12/72-05/04/74	12	33.6	4,887	9.9
5	JP1858	11, 12	02/06/72-12/03/74	20	11.7	1,387	2.8
6	PSR1907+02	8	26/03/73-23/03/74	11	5.4	751	5.7
7	OP1914+13	7, 13	05/09/72-16/01/74	13	20.1	241	0.86
8	PSR1917+00	8	26/03/73-23/03/74	11	3.5	927	5.3
9	MP1944	7, 14	02/06/72-23/03/74	21	16.7	582	570
10	JP1946	11, 15	03/07/72-12/03/74	13	14.7	507	3.2
11	JP1953	11, 12	16/02/73-12/03/74	11	6.9	1,260	3,980 (1,290*)
12	JP2003	7, 12	03/07/72-12/03/74	16	4.9	558	0.90
13	PSR2020+29	7, 16	13/12/72-23/03/74	13	4.4	222	5.7

*Corresponds to maximum dP/dt allowed by error bar.**Table 2** Fitted parameters for thirteen pulsars*

Serial no.	RA h min s	(1950)	Dec.	Epoch (JD-2440000)	Period (A.1s)	dP/dt (10^{-15} s s $^{-1}$)
1	03 01 42.43 0.05		19°21'11.6 2.2	1543.8750	1.387 583 579 433 18	1.294 20 58
2	05 40 06.86 0.09		23 28 15.4 33.8	1708.5625	0.245 965 194 092 10	15.428 78 59
3	06 11 15.59 0.05		22 31 42.0 20.0	1708.6250	0.334 919 019 183 72	59.731 0 4 6
4	09 43 27.24 0.41		10 05 50.0 16.4	1665.8125	1.097 703 639 72 94	3.533 43
5	18 59 01.923 0.014		03 26 46.3 0.6	1470.7500	0.655 444 707 516 46	7.502 0 1 8
6	19 07 07.75 0.05		02 49 55.8 0.6	1767.9375	0.494 913 709 26 24	2.762 14
7	19 15 21.566 0.004		13 48 28.5 0.1	1565.5000	0.194 625 883 200 7	7.201 57 27
8	19 17 17.22 0.01		00 16 03.0 0.3	1767.9375	1.272 255 295 54 13	7.674 8
9	19 44 38.75 0.01		17 58 15.4 0.2	1470.8125	0.440 618 461 656 14	0.0244 5
10	19 46 33.945 0.007		35 32 38.2 0.2	1501.6875	0.717 306 326 897 50	7.054 7 1 4
11	19 52 21.83 0.03		29 15 22.3 0.5	1730.0625	0.426 676 785 45 11	0.003 4 7 1
12	20 02 53.715 0.006		31 28 34.0 0.1	1501.7500	2.111 211 782 83 6	74.581 8 1 8
13	20 20 33.295 0.004		28 44 43.2 0.1	1665.3125	0.343 400 843 442 9	1.895 6 5

*Errors in least significant digits are shown below each parameter.

PSR0611+23 and PP0943. A quantitative study of this effect has not yet been made.

Perhaps the most surprising results of these measurements are the unusually small values of dP/dt for MP1944 and JP1953, leading to the long 'lifetimes' of Table 1. According to our tabulation of dP/dt for 42 pulsars, dP/dt for MP1944 is about one order of magnitude less than the previous smallest value (AP2016— $dP/dt = 0.15 \times 10^{-15}$), and that for JP1953 may be two orders of magnitude smaller. F. D. Drake (personal communication) has suggested that JP1953 may in fact be a white dwarf rather than a neutron star, but at present there seems to be no definitive way to confirm this possibility by calculation. The total radio flux, an essential parameter in testing such a hypothesis, has, unfortunately, not yet been measured for either JP1953 or MP1944. Until more is known about the emission mechanism of pulsars, it will be difficult to decide on theoretical grounds whether or not JP1953 represents a new class of object.

This work was supported in part by the National Science Foundation.

D. W. RICHARDS

Arecibo Observatory,
National Astronomy and Ionosphere Center,
Arecibo, Puerto Rico 00612

J. M. RANKIN

Department of Physics and Astronomy,
University of Iowa, Iowa City, Iowa 52242

G. A. ZEISSIG

Department of Physics,
University of Puerto Rico, Puerto Rico 00931

Received June 28, 1974.

- Rankin, J. M., Counselman, C. C., III, and Richards, D. W., *Astr. J.*, **76**, 686 (1971).
- Richards, D. W., Pettengill, G. H., Counselman, C. C., III, and Rankin, J. M., *Astrophys. J. Lett.*, **160**, L1 (1970).
- Boriakoff, V., thesis, Univ. Cornell (1973).
- Ash, M. E., Shapiro, I. I., and Smith, W. B., *Astr. J.*, **72**, 338 (1967).
- Boynton, P. E., Groth, E. J., Hutchinson, D. P., Nanos, G. P., Partridge, R. B., and Wilkinson, D. T., *Astrophys. J.*, **175**, 217 (1972).
- Colla, G., Salter, C. J., and Sutton, J. M., *IAU Circ. No. 2374* (1971).
- Manchester, R. N., Taylor, J. H., and Huguenin, G. R., *Nature phys. Sci.*, **240**, 74 (1972).
- Davies, J. G., Lyne, A. G., and Seiradakis, J. H., *Nature*, **240**, 229 (1972).
- Graham, D., and Hunt, G. C., *Nature phys. Sci.*, **242**, 85 (1973).
- Vitkevich, V. V., Alekseev, Yu. I., Zhuravlev, V. F., and Shitov,

- Yu. P., *Nature*, **224**, 49 (1969).
¹¹ Manchester, R. N., and Taylor, J. H., *Astrophys. Lett.*, **10**, 67 (1972).
¹² Davies, J. G., Large, M. I., and Pickwick, A. C., *Nature*, **227**, 1123 (1970).
¹³ Swarup, G., Mohanty, D. K., and Balasubramanian, V., *IAU Circ.* No. 2356 (1971).
¹⁴ Vaughan, A. E., and Large, M. I., *Nature*, **225**, 167 (1970).
¹⁵ Davies, J. G., and Large, M. I., *Mon. Not. R. astr. Soc.*, **149**, 301 (1970).
¹⁶ Salter, C. J., *IAU Circ.* No. 2287 (1970).
¹⁷ Manchester, R. N., and Taylor, J. H., *Astrophys. J. Lett.*, **191**, L63 (1974).

Effects of thermal photon scattering on relativistic electrons near pulsars

We assume that pulsar radiation is generated close to the surface of a neutron star. The production of this radiation clearly involves some sort of coherent motion of relativistic electrons, which are presumed to have been accelerated off the stellar surface by large electric fields along magnetic field lines of force. Rengarajan¹ has pointed out that these electrons may scatter against black body photons emitted by the hot neutron star. The hotter the star, the greater is the density of these photons, and the greater the probability that electrons will scatter against them. Our purpose here is to examine the consequences of such collisions and the conditions required for them to take place.

First, we find the limit on the neutron star temperature such that scattering occurs between a thermal photon and a relativistic electron. Let $\mathcal{E}'$ be the energy of a photon in the rest frame of the electron, and $\mathcal{E}$ the corresponding energy in the rest frame of the neutron star. We have $\mathcal{E}' \simeq \gamma \mathcal{E}$ and $\mathcal{E} \simeq 3kT$ where γ is the Lorentz factor of the electron, k is Boltzmann's constant and T is the surface temperature of the star. Defining

$$\chi \equiv (2\mathcal{E}'/mc^2) \simeq (6\gamma kT/mc^2) \quad (1)$$

(where m is the mass of the electron and c the speed of light), the cross section σ for electron-photon scattering is given by the Klein-Nishina formula²

$$\sigma = \begin{cases} \sigma_T & \text{for } \chi \ll 2 \\ 3/4 \sigma_T (\ln \chi + 1/2) & \text{for } \chi \gg 2 \end{cases} \quad (2a)$$

$$\chi \quad (2b)$$

where the Thompson cross section $\sigma_T = 0.66 \times 10^{-24} \text{ cm}^2$. The scattering cross section is Thompson if $\chi \ll 2$; $\chi = 2$ corresponds to $\gamma = \gamma_T \equiv 2 \times 10^9 T^{-1}$. If $\gamma \gg \gamma_T$, the scattering is Compton. The number density of photons within a black body enclosure is $0.244 (kT/hc)^3$, where h is Planck's constant; n , their number density just above the neutron star surface, is half this value. The mean free path λ of an electron is then $\lambda \simeq (n\sigma)^{-1}$ for distances much less than the stellar radius.

Rengarajan estimates the critical temperature in order that mildly relativistic electrons scatter within about one stellar radius R from the surface. With $\gamma \leq 10^3$ the scattering is Thompson or close to it, so that the condition that $\lambda \leq R$ is approximately given by $R \geq (n\sigma_T)^{-1}$. Thus he finds (using a slightly more rigorous method)

$$T \geq R^{-1/3} 10^8,$$

which for $R = 10^6 \text{ cm}$ yields the limit $T \geq 10^6 \text{ K}$, a very reasonable temperature for pulsars. We will show, however, that such estimates are good only if the accelerating electric field strengths are unusually low.

We now consider an electron accelerated by an electric field along a magnetic field line leading away from the surface of a neutron star. If it scatters (against anything) and acquires a component of momentum across the magnetic field, it is then decelerated by the emission of synchrotron radiation. The energy

of such an electron in a medium with aligned electric (E) and magnetic (B) fields has an energy in time³

$$\gamma(t)mc^2 = mc^2 [1 - \beta_\perp^2 (1 - \beta_\parallel^2)^{-1} \exp(-2\Omega_B \delta s)]^{-1/2} \cosh(\Omega_B a s + a) \quad (3)$$

where s is the proper time given by $t = \int_0^s [\gamma(s')]^{-1} ds'$, and $\Omega_B = eB/mc$, $\alpha = E/B$, $\delta = \Omega_B \tau (1 + \alpha^2)$, and $\tau = 3/2 e^2/mc^3$. Here $\cosh a = (1 - \beta_\perp^2)^{-1/2}$, where $c\beta_\parallel$ and $c\beta_\perp$ are the initial velocities parallel and perpendicular to the field. For pulsar parameters $B = 10^{12}$ and $E \ll B$ we have $1 \gg \delta \gg \alpha$; the electron energy drops rapidly to $\mathcal{E}_{\min} = mc^2(1 - \beta_\parallel^2)^{-1/2}$ in a proper time $s \sim (\Omega_B \delta)^{-1}$, and then begins to accelerate parallel to the field.

Suppose first that the scattering is Thompson. In Thompson scattering the energy of the photon is typically increased to $6\gamma^2 kT$. This photon is emitted at an angle $\theta \sim \gamma^{-1}$ to the initial direction of the electron along the magnetic field. The change in perpendicular momentum of the photon is then about $6\theta\gamma^2 kT/c$ or $6\gamma kT/c$. The electron likewise experiences a change of perpendicular momentum $p_\perp \approx 6\gamma kT/c$ and its $\beta_\perp \approx 6kT/mc^2$. This momentum is non-relativistic so that the electron will emit cyclotron radiation at frequency⁴ $\gamma\Omega_B$. From equation (3) we see that it will lose primarily only its perpendicular energy, which is small compared with its total energy, before being reaccelerated. So the electron's motion along the magnetic field line is essentially unaffected by Thompson scattering. (This discussion corrects that of Rengarajan¹, who erroneously concluded that Thompson scattering would drastically affect the electron's motion.)

Secondly suppose that the scattering is Compton. In Compton scattering the scattered photon and electron typically come out with about the same energy ($\sim 1/2 \gamma mc^2$) at an angle $\theta \approx (6kT/\gamma mc^2)^{1/2}$. The electron's initial perpendicular momentum is about $p_\perp = (3/2 \gamma kT/mc^2)^{1/2} mc$ and is relativistic ($\gg mc$). From equation (3) we see that its final energy before being reaccelerated, though relativistic, is now much less than its initial energy:

$$1 \ll \gamma_{\text{final}} \simeq (\gamma mc^2/6kT)^{1/2}$$

For example, with $\gamma = 10^7$ and $T = 10^6$, $\gamma_{\text{final}} \simeq 10^{-2}\gamma$. Because the cross section for scattering (equation (2)) increases as γ decreases, the probability for scattering will again be greatly increased. In fact the electron will Compton scatter again before it can recover its initial energy. In this way the electron's energy will be progressively decreased until $\chi \simeq 2$, after which the scatterings are in the Thompson regime and the electron's energy will be only slightly affected by collisions.

Compton scatterings will therefore limit electron energies in pulsar magnetospheres to $\gamma_T = 2 \times 10^9 T^{-1}$. Many people regard the radio emission from pulsars as curvature radiation. In order for this to be so one requires $\gamma \geq 10^3$, which places an upper limit on the temperatures of the pulsars of about $2 \times 10^6 \text{ K}$. This limit, however, is only relevant if such scatterings do, in fact, take place. This, in turn, is a question which depends on the specific structure of the pulsar magnetosphere, and we have been unable to come to any general conclusions with regard to it. In what follows we will re-examine the conditions for scattering within the context of the pulsar model proposed by Sturrock⁵.

Within Sturrock's model the electric field E increases linearly with distance r above the surface and is given by $E = 3 \times 10^2 r P^{-1}$ statvolt cm^{-1} for $r \leq r_p \equiv 2 \times 10^4 P^{-1/2}$, where P is the pulsar's rotation period in seconds. If the electron accelerates from rest at the surface it will have an energy $\gamma mc^2 = 1/2 Eer$ at a height r , or $\gamma = \gamma_r = 10^{-1} r^2 P^{-1}$. But in fast pulsars with $P \leq 0.7 \text{ s}$, at a distance of $r_L = 3 \times 10^4 P^{4/7}$, the emission of curvature radiation slows the increase of energy, limiting γ to $\gamma_L = 10^7 r^{1/4}$ for $r_L \leq r \leq r_p$. Finally, at heights $r \geq r_p$ the accelerating electric field presumably decreases with a scale height of about r_p . The energy of the electron then rapidly decreases due to the continual emission of curvature radiation. At about one stellar radius the

energy reaches its asymptotic value given by $\gamma \simeq \gamma_\infty = 6 \times 10^7 P^{1/3}$ for fast pulsars and $\gamma \simeq \gamma_{\max} = 3 \times 10^7 P^{-2}$ for slow pulsars ($P \geq 0.7$ s).

The scattering cross section will be Thompson if $\gamma \leq \gamma_T$, that is at heights $r < r_T \equiv 2 \times 10^8 T^{-1/2} P^{1/2}$. The condition that the mean free path $\lambda_T = (\pi \sigma_T)^{-1} \approx 10^{23} T^{-3}$ by less than r_T requires $T > T'$ where $T' = 2 \times 10^7 P^{1/5}$ K. This condition is more severe than that given by Rengarajan, because he required only that $\lambda \leq R \simeq 10^6$ cm. Turning then to the Compton scattering limit $\chi \gg 2$ we have the mean free path

$$\lambda = 4/3 \lambda_T [\chi / (\ln \chi + 1/2)] \quad (4)$$

and $\chi = 6 \times 10^{-11} T r^2 P^{-1}$ for $r_T \leq r \leq r_L$. At $r = r_T$, $\lambda > r$; because $\chi \propto r^2$, λ increases faster than r . Thus $\lambda \gg r$ for $r > r_T$ and electrons will never scatter in this regime. For $r_L \leq r \leq r_P$ in fast pulsars the energy of the electron is ultimately radiation reaction limited, and $\chi = 10^{-2} T r^{1/4}$. The mean free path then increases more slowly than r , but because $\gamma \gtrsim 10^8$ already, $\lambda \leq r_P$ only if $T \geq 10^8$ K. Finally, beyond r_P , γ decreases and the scattering cross section increases. The condition that $\lambda \leq R$ at $r = R$, where R is the stellar radius, is that

$$T^2 \ln(10^{-0.8} P^{1/2} T) \geq 10^{16} P^{1/3}$$

for fast pulsars and that

$$T^2 \ln(10^{-1.3} P^{-2} T) \geq 10^{15.6} P^{-2}$$

for slow pulsars. Thus electrons will Compton scatter at least once if T exceeds $10^{7 \pm 0.2}$ K for $3 \times 10^{-3} \leq P \leq 3$ s.

These limits for both Compton and Thompson scattering lie significantly above the observational upper limits on the temperatures of the known pulsars⁶. The processes under consideration cannot, therefore, be occurring within them. With regard to new pulsars, however, the situation is reversed, for the surface temperature of a newly-formed neutron star is very great. By our analysis such a neutron star will be unable to generate curvature radiation (because of the low limits on γ) until it has cooled to below 10^7 K, a process which may take as long as several years. Thus we conclude that very young neutron stars may not be pulsars.

The above model conditions for scattering are, of course, dependent on the specific spacial structure and strengths of the electric fields that give rise to the radiating electrons. The electric fields may in fact be much weaker than those chosen; thus less stringent (lower) limits on the temperatures are possible. But in stars of low temperatures the photon-electron scatterings will have negligible effect on the coherence properties of the electron stream. Therefore, it is doubtful that thermal photon collisions with the radiating electrons place any theoretical limits on the temperature of known pulsars.

This research has been supported in part by the NSF.

EUGENE TADEMARU

University of Massachusetts,
Amherst, Massachusetts

GEORGE GREENSTEIN

Amherst College,
Amherst, Massachusetts

Received April 8, 1974.

¹ Rengarajan, T. N., *Nature phys. Sci.*, **242**, 102 (1972).

² Jackson, J. D., *Classical Electrodynamics* (John Wiley and Sons, New York, 1962).

³ Mitchell, T. P., Chirinella, J., and Lingerfelt, J. E., *Plasma Phys.*, **13**, 387 (1971).

⁴ Tademaru, E., *Astrophys. J.*, **172**, 327 (1972).

⁵ Sturrock, P. A., *Astrophys. J.*, **164**, 529 (1971).

⁶ Greenstein, G., and McClintock, J., *Science* (in the press).

Dicarboxylic acids in the Murchison meteorite

INVESTIGATIONS of the extractable organic material found in the Murchison meteorite, a carbonaceous chondrite, have led to the identification of many different classes of compounds, including amino acids¹⁻⁵, hydrocarbons^{3,6,7}, heterocycles which contain nitrogen^{8,9}, and monocarboxylic acids¹⁰. We report here a large suite of aliphatic dicarboxylic acids found in extracts from the chondrite. This suite includes many of the branched and straight chained isomers with up to nine carbon atoms. The large number of compounds and the isomeric distribution suggest that, like the amino acids, they are the product of an abiotic synthesis.

The meteorite sample used for this study was prepared by removing the outer surface and reducing the interior piece to a fine powder with a mortar and pestle. A hot water extract of a 2-g sample was obtained by refluxing for 20 h with 6 ml of water. After centrifuging, the supernatant and subsequent wash solution were dried by rotary evaporation and redissolved in 0.5 ml of 0.09N HCl-methanol. (The 0.09 N HCl-methanol solution was prepared by dissolving 0.36 ml of 12 N HCl in 48 ml of absolute methanol.) The resulting solution was applied to a bed of clean, dry silica gel (0.9 cm by 10 cm) and eluted with 0.09 N HCl-methanol. The first 3.0 ml of the eluate was collected, evaporated to dryness, and the residue taken up in 3.0 ml of 1.0 N HCl saturated with sodium chloride. The solution was extracted continuously with diethyl ether for 18 h. The ether extract was divided into three fractions. The first fraction was evaporated to dryness and the di-(+)-2-butyl esters prepared by heating the residue for 1 h at 105° C with 1 ml of absolute (+)-2-butanol which was 8 N in HCl (ref. 11). The second fraction was evaporated to dryness and the *n*-butyl esters prepared by heating the dry residue for 30 min at reflux with 1 ml of absolute *n*-butanol which was 1.25 N in HCl (ref. 12). Similarly, the methyl esters were prepared by reacting the residue of the dry third fraction in methanol, with ethereal diazomethane.

The di-(+)-2-butyl esters were separated by gas chromatography (GC) using a wall coated column of UCON 75 H-90000 (150 feet by 0.02 inch). The column was programmed from 100° C to 155° C at 1° C min⁻¹. The same gas chromatograph was coupled to a mass spectrometer (MS) by means of a membrane separator, for GC/MS investigations. A data system was used to acquire, store, and process the data. The GC and GC/MS studies of the *n*-butyl esters and methyl esters were performed using a gas

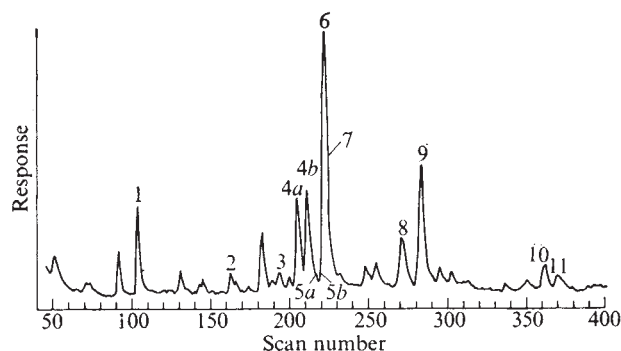


Fig. 1 Total ion plot, reconstructed by computer, of the di-(+)-2-butyl esters of the dicarboxylic acids from the Murchison carbonaceous chondrite 1, Oxalic acid; 2, malonic acid; 3, 2,2-dimethylsuccinic acid; 4a, b, methylsuccinic acid; 5a, b, 2,3-dimethylsuccinic acid; 6, succinic acid; 7, fumaric and/or maleic acid; 8, 3-methylglutaric acid; 9, glutaric acid; 10, adipic acid; 11, 3-methyladipic acid.

chromatograph coupled by means of an all-glass membrane separator¹³ to a mass spectrometer interfaced to a computer system. The GC separations were carried out using a coiled-glass column (6 feet by 0.25 inch) packed with Tabсорb. The column was programmed from 100° C to 210° C at 4° C min⁻¹. Both mass spectrometers were operated at an ionising energy of 70 eV. A procedural blank showed no dicarboxylic acids.

The dicarboxylic acids found in the extract of the chondrite have been identified by their GC retention values and by comparison of their mass spectra with those of authentic standards. Thus, 17 dicarboxylic acids have been identified positively (Table 1). In addition, numerous other GC peaks were observed (Figs 1 and 2), many of which yielded mass spectra suggestive of the aliphatic dicarboxylic acids, but which, at this time, remain unidentified because of a lack of authentic standards.

The presence of a large suite of dicarboxylic acids in carbonaceous chondrites has not been reported previously. The isolated dicarboxylic acids consisted of a number of straight and branched chain isomers with up to nine carbon atoms. All of the structural isomers of the dicarboxylic

Table 1 Dicarboxylic acids found in extracts of the Murchison meteorite

Oxalic acid	Adipic acid
Malonic acid	3-Methyladipic acid
2,2-Dimethylsuccinic acid	Pimelic acid
Methylsuccinic acid	Suberic acid
2,3-Dimethylsuccinic acid	Azelaic acid
Succinic acid	Methylmalonic acid
Maleic and/or fumaric acid	3,3-Dimethylglutaric acid
3-Methylglutaric acid	2,2-Dimethylglutaric acid
Glutaric acid	

acids with two, three and four carbon atoms have been identified, and six of the eight possible structural isomers of the aliphatic dicarboxylic acids with five and six carbon atoms have been identified. Many of the remaining isomers seem to be present; that must be confirmed by comparison with authentic standards. The presence of a large number of isomers of the low molecular weight dicarboxylic acids is analogous to the distribution of amino acids found in this specimen of the Murchison meteorite (Pereira, W. E., *et al.*, unpublished); it is also analogous to the distribution of amino acids and monocarboxylic acids found in other specimens of the Murchison and Murray carbonaceous chondrites^{1-3,10}. The distribution of the aliphatic dicarboxylic acids suggests, therefore, that a random chemical synthesis may be responsible for their presence. The state of all of the dicarboxylic acids in the meteorite sample is not known. Whewellite, the calcium salt of oxalic acid has, however, been isolated and identified¹⁴. Whether all of these compounds occur as salts or whether they are present as free dicarboxylic acids or some type of precursor to the dicarboxylic acids is unknown.

In addition to the saturated dicarboxylic acids, some unsaturated dicarboxylic acids were observed. Mass chromatography has been used to detect an unsaturated dicarboxylic acid (maleic and/or fumaric acid) which elutes with succinic acid during GC analysis. But maleic and fumaric acids have the same GC retention values on the columns which we used, and their mass spectra are virtually identical, so we were unable to distinguish between these two dicarboxylic acids in this investigation.

In addition to the large number of structural isomers found in this sample, some of the dicarboxylic acids with asymmetrical centres show a GC separation of their enantiomers (see Fig. 1). Although the separation of the optical isomers of amino acids has been accomplished by the preparation of the diastereoisomeric (+)-2-butyl derivatives,

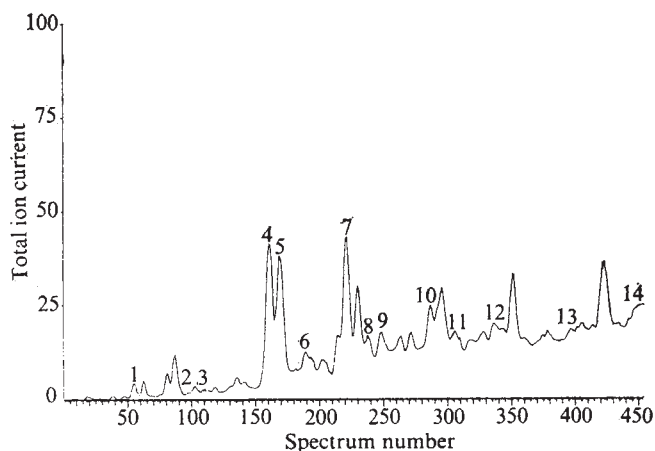


Fig. 2 Total ion plot, reconstructed by computer; of the di-*n*-butyl esters of the dicarboxylic acids extracted from the Murchison carbonaceous chondrite. 1, Oxalic acid; 2, methylmalonic acid; 3, malonic acid; 4, succinic and maleic or fumaric acid; 5, methylsuccinic acid; 6, 2,3-dimethylsuccinic acid; 7, glutaric acid; 8, 3-methylglutaric acid, and 3,3-dimethylglutaric acid; 9, 2,2-dimethylglutaric acid; 10, adipic acid; 11, 3-methyladipic acid; 12, pimelic acid; 13, suberic acid; 14, azelaic acid.

similar separation of the optical isomers of the dicarboxylic acids as the (+)-2-butyl diesters has not been reported previously. A detailed investigation of the appropriate conditions, reagents, GC columns, and so on, necessary to derive these products, is in progress and will be reported in the future. It should be noted that, in the manner analogous to amino acids obtained from abiotic sources, both enantiomers of R and S-methyl succinic acid seem to be present in nearly equal concentration (see Fig. 1). A similar finding has been reported for a stimulated, abiotic, chemical evolution experiment¹⁵. The continued investigation of the dicarboxylic acids is important because, apart from amino acids, they are the only other compounds so far found in meteorites in which the optical isomers have been separated. The aliphatic dicarboxylic acids thus represent a potential suite of marker compounds for distinguishing between indigenous nonbiological and biological compounds found in extraterrestrial samples. In addition, they are present in extracts of the Murchison meteorite at concentrations approximately one to two orders of magnitude higher than the amino acids. The exact quantity and distribution of aliphatic dicarboxylic acids, when compared with similar information for amino acids and fatty acids, should provide insight into the organic reactions responsible for their presence in carbonaceous chondrites.

We thank the Smithsonian Institution for providing the sample (5455) used in this study. The research performed by W.E.P., R.E.S., and A.M.D. was supported financially by NASA.

J. G. LAWLESS
B. ZEITMAN

Planetary Biology Division,
Ames Research Center,
Moffett Field, California 94035

W. E. PEREIRA
R. E. SUMMONS
A. M. DUFFIELD

Department of Genetics,
Stanford University Medical Center,
Stanford, California

Received March 28, 1974.

¹ Kvenvolden, K., *et al.*, *Nature*, **228**, 923 (1970).

- ² Kvenvolden, K., Lawless, J., and Ponnamperna, C., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 486 (1971).
- ³ Oro, J., Gibert, J., Lichtenstein, H., Wikstrom, S., and Flory, D. A., *Nature*, **230**, 105 (1971).
- ⁴ Lawless, J. G., *Geochim. cosmochim. Acta.*, **37**, 2207 (1973).
- ⁵ Cronin, J. R., and Moore, C. B., *Science*, **172**, 1327 (1971).
- ⁶ Pering, K. L., and Ponnamperna, C., *Science*, **173**, 237 (1972).
- ⁷ Studier, M. H., Hayatsu, R., and Anders, E., *Geochim. cosmochim. Acta*, **36**, 189 (1972).
- ⁸ Folsome, C. E., Lawless, J. G., Romiez, M., and Ponnamperna, C., *Nature*, **332**, 108 (1972).
- ⁹ Folsome, C. E., Lawless, J. G., Romiez, M., and Ponnamperna, C., *Geochim. cosmochim. Acta*, **37**, 455 (1973).
- ¹⁰ Yuen, G., and Kvenvolden, K. A., *Nature*, **246**, 301 (1973).
- ¹¹ Pollock, G. E., *Analyt. Chem.*, **44**, 2368 (1972).
- ¹² Roach, D., and Gehrke, C. W., *J. Chromatogr.*, **44**, 269 (1969).
- ¹³ Hawes, J. E., Mallaby, R., and Williams, V. P., *J. chromatogr. Sci.*, **7**, 690 (1969).
- ¹⁴ Fuchs, L. H., Olsen, E., and Jensen, K. J., *Smithsonian Contributions to the Earth Sciences*, Number 10, (1973).
- ¹⁵ Zeitman, B., Chang, S., and Lawless, J. G., *Nature*, **251**, 42 (1974).

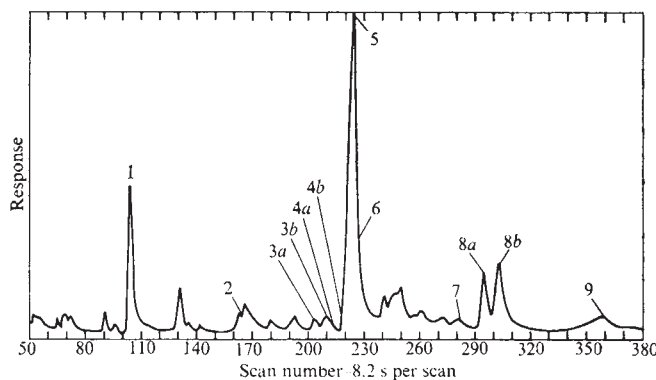


Fig. 1 Gas chromatogram, reconstructed by computer, of the di-(+)-2-butyl esters of the dicarboxylic acid products of electrical discharge. Identifying numbers as in Table 1. A wall-coated column of UCON 75 H 90000 (150 feet by 0.02 inches) was temperature programmed from 100° C to 150° C at 1° C min⁻¹. The helium flow was 5 ml min⁻¹.

Dicarboxylic acids from electric discharge

OPARIN¹ and Urey² have suggested that methane, ammonia, hydrogen and water were present in the primitive atmosphere of the Earth. Significant yields of amino, hydroxy, and monocarboxylic acids have been produced by the action of electric discharge on such a primordial gas mixture^{3,4}; and numerous chemical evolution experiments have produced many types of biologically significant molecules⁵. Apart from the detection of succinic acid^{3,4,6}, no other dicarboxylic acids have been reported in chemical evolution experiments.

A large suite of dicarboxylic acids, indigenous to the Murchison meteorite, have been identified, and they were probably produced in a random, nonbiological synthesis⁷. As a result of this discovery, the possible synthesis of a similar suite of dicarboxylic acids was investigated in a chemical evolution experiment which simulated electric discharge through the primitive atmosphere of the Earth. Among the products of this experiment, saturated, unsaturated, methyl and chloro-substituted dicarboxylic acids were detected, using gas chromatography (GC) and mass spectrometry (MS).

A 10-l electric discharge chamber equipped with four Tesla coils was used (see ref. 8). A reaction mixture of 200 mm CH₄, 200 mm N₂, and 400 ml NH₄Cl solution (0.05 M, pH 8.77) was sparked for 48 h (ref. 9). The aqueous phase was rendered strongly basic with NH₄OH, then filtered and stored in a freezer until the analysis. Fifteen millilitres of this filtrate was concentrated to 5 ml by freeze-drying. For the analysis, 0.5 ml of the concentrated filtrate was evaporated to dryness and redissolved in 0.5 ml of 0.09 N HCl in methanol (prepared by adding 0.36 ml 12 N HCl to 48 ml of absolute methanol). This solution was applied to a dry column (0.9 cm by 10 cm) of Adsorbosil-2, a silica gel without binder. This adsorbent was batch-washed seven times with 0.1 N HCl in methanol, then dried and equilibrated to room humidity before use. After the application of the sample, a 10-cm head of 0.09 N HCl in methanol was placed over the column and the chromatography was begun. When the solvent reached the bottom of the column, nitrogen pressure was applied and fractions of 0.5 ml were collected. The fractions were monitored using thin-layer chromatography. The first 2.5 ml (containing the amino acids and dicarboxylic acids) were centrifuged to remove silica gel fines and then evaporated to dryness. The residue was dissolved in 1 N HCl saturated with NaCl. This solution was continuously extracted with diethyl ether for 16 h (to separate the amino acids from the dicarboxylic acids). The ether extract was dried, and diester derivatives of the dicarboxylic acids were prepared by heating the residue for 1 h, under anhydrous conditions, with (+)-2-butanol which was 5 N in HCl. Excess reagent was evaporated with a stream of

dry, filtered nitrogen. The di-(+)-2-butyl esters of the diacid were dissolved in 10 μ l of methylene chloride. A 2- μ l sample of this solution (equivalent to 0.25 ml of the original electric discharge aqueous phase) was subjected to analysis by GC/MS. A gas chromatograph was coupled to a mass spectrometer (operated at 70 eV) by means of a membrane separator. A data system was used to acquire, store, and process the data. No dicarboxylic acids were detected in the procedural blank. Identification of the derivatised diacid electric discharge products is based on laboratory comparisons of their mass spectra with those of authentic standards. Further confirmation was obtained by comparing gas chromatographic retention times of the products and standard compounds by co-injection techniques. No significant racemisation was observed when R-methylsuccinic acid was run through the analytical procedure.

Figure 1 shows the gas chromatographic separation of the derivatives of the diacids produced by spark discharge. Table 1 lists those compounds that were confirmed by correlation of both chromatographic retention times and mass spectra with the corresponding authentic standards. Mass chromatography was used to detect fumaric and/or maleic acid which elute with succinic acid. The characteristic *m/e* 157 for succinic acid and *m/e* 155 for fumaric and maleic acid were monitored (Fig. 2). These mass fragments correspond to the fragments of the respective derivatives minus one butoxy group. Fumaric and maleic acid have identical retention times under the chromatographic conditions used and their mass spectra are virtually the same. In a similar manner, mass chromatography revealed two peaks for the three possible configurational isomers of 2,3-dimethylsuccinic acid. The use of optically active (+)-2-butanol to prepare diester derivatives of the diacids was necessary for the separation of those compounds containing asymmetric carbon atoms. Enantiomeric pairs, such as R,S-methylsuccinic acid and R,S-2-chlorosuccinic acid, were separated using gas chromatography, with good resolution. The exact order of elution of the enantiomers is under investigation in our laboratory, subject to the availability of pure, optically active standards.

Table 1 Dicarboxylic acids identified as products of electric discharge

1	Oxalic acid	5	Succinic acid
2	Malonic acid	6	Fumaric and/or maleic acid
3a	Methylsuccinic acid*	7	Glutaric acid
3b	Methylsuccinic acid*	8a	2-Chlorosuccinic acid*
4a	2,3-Dimethylsuccinic acid*	8b	2-Chlorosuccinic acid*
4b	2,3-Dimethylsuccinic acid*	9	Adipic acid

* Elution order of configurational isomers is uncertain.

Five of the eight possible structural isomers of the non-chlorine containing aliphatic dicarboxylic acids with two to five carbon atoms have been detected. Furthermore, many of the unidentified peaks (Fig. 1) have mass spectra suggestive of dicarboxylic acids. Their identification is subject to the availability of pure standards. The variety of structural isomers found is not surprising, because similar arrays of isomers arising from random chemical synthesis have been detected for hydrocarbons, amino acids, and monocarboxylic acids derived from chemical evolution studies⁵.

The suite of dicarboxylic acids in the electric discharge experiment is similar to that of the Murchison meteorite, except for the fact that 2-chlorosuccinic acid is present in the spark discharge. Because NH_4Cl was used in the experiment, chloro-acids were probably formed by the interaction of the discharge with aerosol particles suspended in the gas phase, or by interaction of chloride ions with reactive intermediates in the liquid phase, or both.

Apart from work with amino acids⁹, this gas chromatographic separation of R,S isomers represents the only case in which molecules possessing asymmetric carbon atoms synthesised in chemical evolution experiments were resolved chromatographically. This gas chromatographic capability seems to provide a rapid, routine, new tool for the differentiation of biologically and nonbiologically produced dicarboxylic acids.

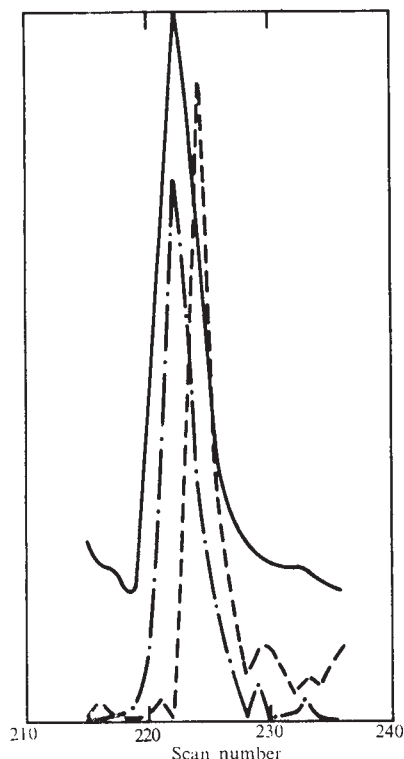


Fig. 2 Mass chromatogram, reconstructed by computer, of a portion of the GC/MS run shown on Fig. 1. Solid curve, total ion current (scaling factor=1); dashed curve, $m/e=155$ (scaling factor=128); — · — ·, $m/e=157$ (scaling factor=16).

Dicarboxylic acids are important metabolic intermediates, especially with respect to photosynthesis and bioenergetics (as in Krebs cycle). The discovery that these compounds can be produced by spark discharge supports the view that dicarboxylic acids were probably present in the prebiotic *milieu*. Thus, the abiotic synthesis of this class of molecules has implications for chemical evolution. Because similar arrays of dicarboxylic acids, as well as amino acids^{9,10}, were found in the Murchison meteorite and the electric discharge experiments, it is possible that a high energy process such as electric discharge, played an important role in the production of the organic matter

found in meteorites. To explore this relationship, we are engaged in comparative studies of the organic and stable isotope chemistry of the meteoritic and synthetic products. Such studies may shed light on the conditions of, and mechanisms involved in, organic synthesis in the early Solar System.

We thank Steven Nitenson, College of San Mateo, for running the electric discharge experiments.

BEN ZEITMAN
SHERWOOD CHANG
JAMES G. LAWLESS

Chemical Evolution Branch,
Planetary Biology Division,
Ames Research Center, NASA,
Moffett Field, California 94035

Received March 28, 1974.

- ¹ Oparin, A. I., *The Origin of Life* (Dover Publications, New York, 1953).
- ² Urey, H. C., *Proc. natn. Acad. Sci. U.S.A.*, **38**, 351 (1952).
- ³ Miller, S. L., *Biochim. biophys. Acta*, **23**, 480 (1957).
- ⁴ Miller, S. L., and Urey, H. C., *Science*, **130**, 245 (1959).
- ⁵ Lemmon, R. M., *Chem. Rev.*, **70**, 95 (1970).
- ⁶ Lawless, J. G., and Boynton, C. D., *Nature*, **243**, 405 (1973).
- ⁷ Lawless, J. G., *et al.*, *Nature*, **251**, 40 (1974).
- ⁸ Miller, S. L., *J. Am. chem. Soc.*, **77**, 2351 (1955).
- ⁹ Ring, D., Wolman, Y., Friedmann, N., and Miller, S. L., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 765 (1972).
- ¹⁰ Lawless, J. G., *Geochim. cosmochim. Acta*, **37**, 2207 (1973).

Vertical mixing in the deep ocean

SEVERAL authors¹⁻³ have reported *in situ* observations of light scattering intensity in the deep ocean, which reveal the widespread occurrence of an ~1 km thick layer of suspended mineral and organic matter above the ocean floor. The thickness of this nepheloid layer is several orders of magnitude greater than the characteristic thickness of the turbulent Ekman layer at the ocean floor⁴. Analysis of observations of the nepheloid layer in the western North Atlantic indicates that an average vertical mixing coefficient, A , ~100 $\text{cm}^2 \text{s}^{-1}$ is required to maintain the layer² in the lowest kilometre or so of the ocean. This value is significantly greater than the traditional ($1 \text{ cm}^2 \text{s}^{-1}$) estimate based on water mass analysis^{5,6} of vertical eddy diffusivity in the deep ocean.

Here, I point out that this rather large mixing coefficient is consistent with mixing induced by internal waves^{6,7} in the lowest kilometre or so of the ocean. The relatively high vertical mixing rates in that region can be accounted for by the peculiarities of the generation of internal waves as a result of the interaction of topographic Rossby waves with relatively small scale ($\leq 1 \text{ km}$) topographic features on the ocean floor.

Following Bell⁸, the rate of energy production in internal waves, E , arising from the flow of a steady uniform current, of speed, U , over a rough bottom, may be approximated as

$$E \sim 2 \rho f N U H^2$$

where ρ is seawater density, f is the Coriolis parameter, N is the representative Brunt-Väisälä frequency, and H is the contribution to the r.m.s. elevation of the topography in the internal wave passband of wave numbers, k , such that $f < kU < N$. In fact, deep ocean currents are not steady, being composed for the most part of tidal and inertial period motions and quasigeostrophic motions with periods of a week or longer⁹. It is this latter component of flow which is relevant here. The characteristic time scale for such motions is sufficiently large that they may be considered as steady as far as processes of internal wave generation are concerned.

Swallow¹⁰ has reported the results of a series of current measurements made during a period of 14 months in the western North Atlantic. The observed structure of the currents may be interpreted in terms of bottom trapped topographic Rossby wave modes¹¹, with velocity decaying on a vertical scale of several kilometres, from a near bottom maximum of roughly 10 cm s^{-1} . The presence of significant topographic structuring on horizontal scales of 10 km and smaller¹² should introduce further variability in the basic current field in the lowest kilometre. The interaction of this topographically modified quasisteady current field with the smaller scale topographical features is responsible for the generation of internal wave energy. As the internal waves propagate away from the bottom, the relative frequency, kU , decays with height. That has serious implications as far as the internal wave field is concerned. The ultimate catastrophe for any given wave component occurs when it encounters a critical level^{13,14} at which the relative frequency is reduced to the local inertial frequency, f . Nonlinear effects inevitably destroy the internal wave component in the vicinity of such a critical level, so that as a consequence of the general decay of background current speed with distance above the bottom, a substantial portion of the internal wave energy should be dissipated in the lowest kilometre or so of the ocean.

The rate of production of internal wave energy may be estimated for conditions representative of the western North Atlantic. A typical deep current speed is, $U \sim 10 \text{ cm s}^{-1}$. A representative value for the Brunt-Väisälä frequency is¹⁵ $N \sim 7 \times 10^{-4} \text{ s}^{-1}$. Analysis of a large number of topographical profiles of abyssal hills in the eastern North Pacific⁶ suggests that a value of $H^2 \sim 10^3 \text{ m}^2$ is representative, and consistent with the results of an analysis of sonic sections¹⁶ in the North Atlantic. At a latitude of 30° N ($f \sim 7 \times 10^{-5} \text{ s}^{-1}$), then, $E \sim 10 \text{ erg cm}^{-2} \text{ s}^{-1}$. If it is assumed that 50% of that energy goes into mixing in the lowest kilometre of the ocean, then there must be an associated buoyancy flux, F , $\sim 5 \times 10^{-3} \text{ cm}^2 \text{ s}^{-3}$. With a Brunt-Väisälä frequency, N , $\sim 7 \times 10^{-4} \text{ s}^{-1}$, that implies a vertical mixing coefficient ($A = F/N^2$) of $\sim 100 \text{ cm}^2 \text{ s}^{-1}$. That is precisely of the same order as the vertical mixing coefficient based on the analysis of observations of the western North Atlantic nepheloid layer². Further support of the proposed relationship between mixing induced by internal waves and the nepheloid layer is provided by the apparent correlation between bottom roughness and thickness of the nepheloid layer³. The mixing induced by internal waves discussed here must be balanced in the overall thermohaline energy budget. Acting over 50% of the North Atlantic, the vertical mixing estimate made here implies a rate of energy conversion of the order of $10^{18} \text{ erg s}^{-1}$. In this connection, Wright¹⁷ has investigated the rate at which available potential energy is released by the sinking of cold water masses in the northern North Atlantic. He found that the two Norwegian Sea overflows, and winter convection in the Labrador Sea, contributed $\sim 0.85 \times 10^{18} \text{ erg s}^{-1}$. The North Atlantic seems to be somewhat unique in the intensity of its deep currents. Near-bottom currents of 3 or 4 cm s^{-1} are probably more representative of the world ocean⁴, so that an estimate of E , $\sim 1 \text{ erg cm}^{-2} \text{ s}^{-1}$, with a corresponding mixing coefficient, A , of the order of $10 \text{ cm}^2 \text{ s}^{-1}$ may be considered as more representative of the world ocean.

THOMAS H. BELL, JUN.

Physical Oceanography Branch,
Ocean Sciences Division,
Naval Research Laboratory,
Washington, DC, 20375

Received April 19; revised July 12, 1974.

- ¹ Eittrheim, S., Gordon, A. L., Ewing, M., Thorndike, E. M., and Burchhausen, P., in *Studies in Physical Oceanography*, vol. 2 (edit. by Gordon, A. L.), 19 (Gordon and Breach, New York, 1972).
- ² Eittrheim, S., and Ewing, M., *ibid.*, 123.
- ³ Connary, S. D., and Ewing, M., *ibid.*, 169.
- ⁴ Wimbush, M., and Munk, W., in *The Sea*, 4, part 1 (edit. by Maxwell, A. E.), 731 (Interscience, New York, 1970).
- ⁵ Sverdrup, H. U., Johnson, M. W., and Fleming, R. H., *The Oceans*, Table 65 (Prentice Hall, Englewood Cliffs, 1942).
- ⁶ Munk, W. H., *Deep Sea Res.*, 13, 707 (1966).
- ⁷ Garrett, C., and Munk, W., *Deep Sea Res.*, 19, 823 (1972).
- ⁸ Bell, T. H., thesis, Johns Hopkins Univ., Baltimore (1973).
- ⁹ Rhines, P., *Boundary Layer Meteorol.*, 4, 345 (1973).
- ¹⁰ Swallow, J. C., *Phil. Trans. R. Soc.*, A270, 451 (1971).
- ¹¹ Rhines, P., *ibid.*, 461.
- ¹² Swallow, *ibid.*, 452.
- ¹³ Booker, J. R., and Bretherton, F. P., *J. Fluid Mech.*, 27, 513 (1967).
- ¹⁴ Jones, W. L., *J. Fluid Mech.*, 30, 439 (1967).
- ¹⁵ Reid, J. L., and Lynn, R. J., *Deep Sea Res.*, 18, 1063 (1971).
- ¹⁶ Cox, C., and Sandstrom, H., *J. oceanogr. Soc. Japan*, 20, 499 (1962).
- ¹⁷ Wright, C. R., *Deep Sea Res.*, 19, 865 (1972).

Thermonuclear micro-explosions with intense ion beams

HIGH density pellet compression may be important in laser fusion¹. In this way the minimum energy input for break-even can be substantially reduced to levels within reach of expected future laser technology. Further² when this concept is applied to a fissionable pellet surrounded by a small neutron reflector, preferably TD, which is compressed together with the reflector, to high densities, very small critical masses become possible; in addition the fission process could ignite a thermonuclear reaction in the TD reflector. In these proposals very large laser energies of $\sim 1 \text{ MJ}$, which may be difficult to reach, are required. As an alternative to laser fusion I have proposed the use of intense relativistic electron beams³, but here the beam pulse length is longer than for laser beams, and the electron stopping range may be too long.

I present here an alternative idea based on the possibility of generating intense ion beams in a magnetically insulated diode^{4,5}. In such a diode a pulsed laser beam is directed on to the anode surface as a high voltage is applied to the diode, and a magnetic field directed parallel to the cathode surface prevents the flow of electrons from the cathode to the anode, but not the flow of ions from the anode to the cathode, provided that the magnetic field is strong enough to make the electron Larmor radius, but not the ion Larmor radius, small compared with the anode-cathode gap. The laser beam hitting the anode surface will produce a surface plasma from which the ions are drawn and accelerated towards the cathode. After their passage through the cathode, which has the form of a grid to minimise the energy losses by collision, the ions are projected into a drift tube. This drift tube is filled with a tenuous plasma of high electrical conductivity. Because of their large inertia the ions draw into their flow electrons which pass through the highly conducting plasma from the cathode surface or the inner surface of the drift tube. They thus compensate for an electric space charge field which would otherwise build up. Furthermore, because of their small mass and high mobility, the electrons will follow the ion flow with the same velocity, thus also cancelling the self-magnetic field of the ion beam entering the drift tube. The ion beam therefore drifts freely as an electrically and magnetically neutral beam.

Calculations for magnetically insulated diodes pulsed with several megavolt predict current densities of the order $j \sim 3 \text{ kA cm}^{-2}$ such that for an area of $\sim 30 \text{ cm}^2$ a total current of $I \sim 10^5 \text{ A}$ could be produced⁵. To deliver a total energy of $\sim 1 \text{ MJ}$ with a voltage of $V \sim 10^7 \text{ V}$ and current of $I \sim 10^5 \text{ A}$ the total discharge time would be $\tau \sim 10^{-6} \text{ s}$. In order to take full advantage of the low ion velocities we may choose singly

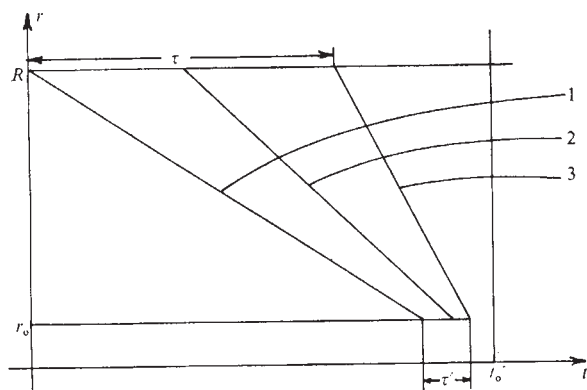


Fig. 1 Time focusing of the beam.

ionised heavy ions, such as ions of lead or uranium, with which the anode surface would have to be made or coated. For a voltage V and atomic weight A , the ion velocity is given by $v \approx 1.38 \times 10^6 \sqrt{(V/A)} \text{ cm s}^{-1}$. Putting $A=200$ gives $v \approx 10^5 \sqrt{V} \text{ cm s}^{-1}$. For $V = 10^6 \text{ V}$, $v \approx 10^8 \text{ cm s}^{-1}$ and for $V = 10^7 \text{ V}$, $v \approx 3 \times 10^8 \text{ cm s}^{-1}$. I propose that this variation of the ion velocity with the voltage may be used to focus the beam by increasing the voltage at the diode during the pulse. Assume for example, that the pulse lasts $\approx 0.7 \times 10^{-6} \text{ s}$ and that an ion at the beginning of the pulse has a velocity of $\approx 10^8 \text{ cm s}^{-1}$, as compared with another ion at the end of the pulse with a velocity of $\approx 3 \times 10^8 \text{ cm s}^{-1}$. One can then easily calculate that after the time $\approx 10^{-6} \text{ s}$ the ion emitted at the end of the pulse would have caught up with the ion emitted at the beginning of the pulse, at a distance of $\approx 100 \text{ cm}$ and time $\approx 3 \times 10^{-7} \text{ s}$ after the end of the pulse. So by shaping the time behaviour of the voltage pulse properly at the diode one can make the ions converge at the same time and place, shortening the beam pulse and raising the beam power by perhaps many orders of magnitude.

By making the ion source spherically symmetric and by projecting a beam of spherical symmetry towards a common centre of convergence, the total beam power density can be further increased. This could be approximated by a large number of diodes in a spherically symmetric arrangement, with the diodes having a concave shape so that each beam pulse would have the form of a spherical segment.

Figure 1 shows how the ion velocity would have to be changed in time during the pulse to focus the beam at time $t = t_0$ and at the common centre of convergence $r = 0$. The ions are emitted at the position $r = R$. The lines 1, 2 and 3 represent the $r - t$ diagram for the initial and intermediate and the final ion of the pulse. Time focusing of the beam then requires for the ion velocity to follow

$$v = R/(t_0 - t) \quad (1)$$

If a target to be bombarded by the beam is placed at $r = r_0$, the pulse length at the target τ' (at which $r = r_0$) is then shortened in comparison to the pulse length at the source (at which $r = R$) by the factor $\tau'/\tau \approx R/r_0$.

It follows that for an ion velocity of v and a pulse length τ a linear beam can be focused in time after a distance $x \sim v\tau$, with a beam energy $E \sim IVx/v$. For $I \sim 10^5 \text{ A}$, $V \sim 10^7 \text{ V}$ and $v \sim 10^8 \text{ cm s}^{-1}$ it follows that $E \sim 10^4 x \text{ J}$. Therefore, for $x = 10^2 \text{ cm}$, with $\tau \sim 10^{-6} \text{ s}$ one would have $E \sim 1 \text{ MJ}$ and for $x = 10^4 \text{ cm}$ with $\tau \sim 10^{-4} \text{ s}$ one would have $E \sim 100 \text{ MJ}$. A drift tube 100 m long is still well within the technical possibilities and the energy of 100 MJ to be pumped into the beam could probably be supplied by an inductive energy storage system. But the energy stored in the drift tube can be delivered in a much shorter time depending on the perfection of the time focusing, which depends on the velocity spread of the ions. Since the ions are produced from the anode material by a

pulsed laser beam, two types of velocity spreads will occur: first, by a differing degree of ionisation; second, by the initial thermal motion. It is conceivable that by a properly chosen laser frequency a high degree of uniform ionisation can be achieved. The most important velocity spread therefore results from the initial thermal ion energy at the anode surface. This energy is of the order 1 eV. In the anode-cathode gap the ions are then accelerated by a voltage of $\sim 10^6$ – 10^7 V , which for singly ionised ions implies an energy of $\sim 10^6$ – 10^7 eV . Therefore, the beam will be monoenergetic to the order $\sim 10^{-6}$ to 10^{-7} corresponding to a relative velocity spread of $\sim 10^{-3}$ to $10^{-3.5}$. Thus, a time focusing of the beam by the same factor will be possible. This would imply, for example, that in the case of the 100 MJ beam the energy could be delivered in a time of about $\sim 10^{-7}$ to $3 \times 10^{-8} \text{ s}$, thus raising the beam power to $3 \times 10^{15} \text{ W}$.

Because of the pulse shortening by time focusing which is possible because of the low ion velocities, energy can be pumped into the beam slowly by inductive energy storage devices pulsing the diode. Since the peak voltage is several million volts, this might be done with a cascade of pulse transformers.

The range of heavy ions in dense matter is rather small (typically $\sim 10^{-4} \text{ cm}$), so the beam can be easily stopped if projected on to a solid piece of fusible (or fission-fusion hybrid) material. Furthermore, by properly shaping the time behaviour of the laser light impinging on the anode, the ion current and thus the beam power can be changed with time. This is of importance if one wants to shape the beam pulse as has been proposed by Nuckolls *et al.*¹ for adiabatic high density pellet compression with a power profile approximately given by

$$\varepsilon = \text{const.} (1 - t/t_0)^{-15/8} \quad (2)$$

It follows that the same power profile can be obtained with

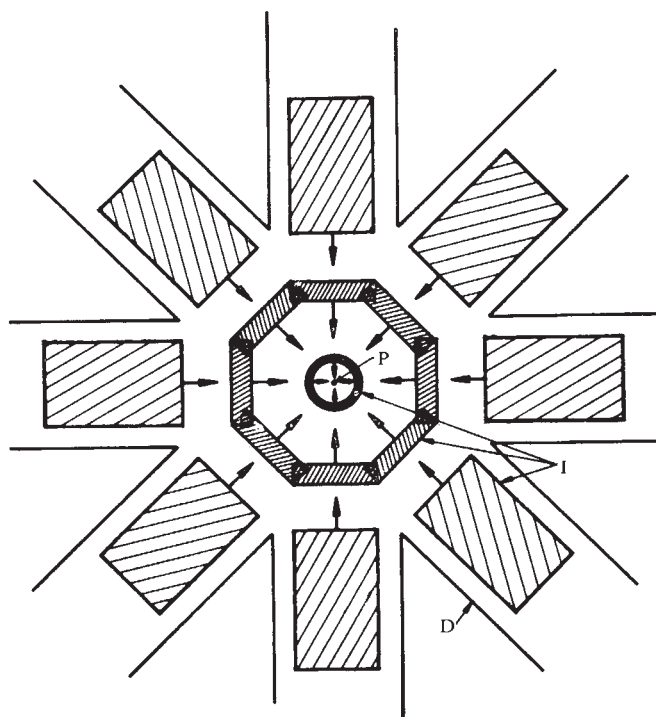


Fig. 2 Coalescence and projection of several ion beams on to a thermonuclear pellet. I, ion beam; D, drift tube; P, thermonuclear pellet.

the proposed ion beam technique. If the accelerating voltage is V and the ion current I then

$$v = \text{const.} \sqrt{V} \quad (3)$$

$$\epsilon = IV \quad (4)$$

From equations (1-4)

$$v = \text{const.} (1 - t/t_0)^{-1} \quad (5)$$

$$V = \text{const.} (1 - t/t_0)^{-2} \quad (6)$$

$$I = \epsilon/V = \text{const.} (1 - t/t_0)^{1/2} \quad (7)$$

Since the ion current is approximately proportional to the laser light intensity, a simultaneous change in both the accelerating voltage and laser light intensity can produce the power profile equation (2).

The proposed technique offers two possibilities for nuclear micro-explosions:

First, the ignition of rather large micro-explosions with two beams of ~ 100 MJ produced in two ~ 100 m drift tubes clashing head on, compressing and heating between them a sandwich of dense thermonuclear or fission-fusion hybrid material. For fusion pellets a minimum energy of $\sim 10^2$ – 10^3 MJ and for hybrid fission-fusion pellets somewhat less energy would be here required. It is therefore conceivable that two beams each having an energy of ~ 100 MJ would suffice.

Second, the use of many beams approximating one beam of spherical symmetry and which are projected simultaneously into a spherical combustion chamber as shown in Fig. 2. There, after having entered the chamber, the different beam pulses would coalesce into one ion pulse having the shape of a spherical shell, the thickness of which would decrease with time as the pulse approaches the centre of convergence at which the pellet to be ignited is placed. For reactor conditions a total beam energy of ~ 1 MJ would be required, as in the case of laser fusion, but since the focusing here may not be as perfect as for optical radiation a larger input energy may be needed; this does not, however, seem to present a problem since the energy can be drawn from cheap inductive storage devices. The pulse length required for solid pellet heating is of the order $\sim 10^{-9}$ s and less. If the pellet has the form of a spherical shell the pulse can be somewhat longer. So if the pulse is delivered from the diode in about $\sim 10^{-8}$ s it has to be compressed by a factor $\sim 10^3$, shortening the pulse length down to $\sim 10^{-9}$ s. This implies a radial beam compression from an initial beam width of ~ 1 m down to ~ 1 mm. It seems that there is a fairly good chance that this can be done.

F. WINTERBERG

Desert Research Institute,
University of Nevada System,
Reno, Nevada 89507

Received April 22, revised June 20, 1974.

¹ Nuckolls, J., *et al.*, *Nature*, **239**, 139 (1972).

² Winterberg, F., *Nature*, **241**, 449 (1973).

³ Winterberg, F., *Phys. Rev.*, **174**, 212 (1968).

⁴ Winterberg, F., in *Physics of High Energy Density*, 370 (Academic Press, New York, 1971).

⁵ Sudan, R. N., and Lovelace, R. V., *Phys. Rev. Lett.*, **31**, 1174 (1973).

0.13×10^{-9} , and a systematic uncertainty of $\pm 0.7 \times 10^{-9}$. (The systematic uncertainty is less than that published previously, because only the reproducibility of the CO_2 stabilised frequency is considered, rather than the uncertainty of obtaining the unperturbed R(12) transition frequency.) The wavelength was determined using interferometric measurements on up-converted light at $0.68 \mu\text{m}$ and was related to the ^{86}Kr primary length standard, realised in accordance with the latest recommendations of the Comité Consultatif pour la Définition du Mètre (CCDM). The value obtained³ for the free space vacuum wavelength is $\lambda = 9,317,246.348 \text{ pm}$, with $\sigma_m = 1.14 \times 10^{-9}$ and a systematic uncertainty of $\pm 1.4 \times 10^{-9}$.

The product of these results gives the value for the speed of light

$$c = 299,792,459.0 \pm 0.8 \text{ m s}^{-1}$$

The uncertainty (corresponding to $\pm 2.7 \times 10^{-9}$) is the arithmetic sum of the standard error of the mean (1.1×10^{-9}) and the total systematic uncertainty ($\pm 1.6 \times 10^{-9}$). Each of these was derived by quadrature summation of the contributions from the frequency and wavelength measurements.

Our measurement may be compared with values derived from a frequency measurement, by Evenson *et al.*⁴, of the methane stabilised He-Ne laser at $3.39 \mu\text{m}$, and the wavelength measurements made in that and other laboratories. The available data was reviewed by the CCDM in June 1973, when the method of realising the metre through the ^{86}Kr source was reconsidered. It was decided that where asymmetry of the spectral profile is observed, the defined wavelength applies to a point midway between the peak and centre of gravity. Thus, the result of Evenson *et al.* becomes: $c = 299,792,457.4 \pm 1.1 \text{ m s}^{-1}$. On consideration of wavelength measurements in four laboratories, however, the CCDM recommended⁵ for general use, the value $c = 299,792,458 \text{ m s}^{-1}$, with an uncertainty of $\pm 4 \times 10^{-9}$ resulting from the uncertainties of the wavelength measurements.

The satisfactory agreement of our result with those based upon the $3.39 \mu\text{m}$ laser confirms both the recommended value for c and the reliability of frequency and wavelength measurement techniques involving infrared laser radiations.

T. G. BLANEY
C. C. BRADLEY
G. J. EDWARDS
B. W. JOLLIFFE
D. J. E. KNIGHT
W. R. C. ROWLEY
K. C. SHOTTON
P. T. WOODS

National Physical Laboratory,
Teddington, Middlesex, UK

Received May 17; revised July 2, 1974.

¹ Bradley, C. C., Edwards, G. J., Knight, D. J. E., Rowley, W. R. C., and Woods, P. T., *Phys. Bull.*, **23**, 15 (1972).

² Blaney, T. G., *et al.* *Nature*, **244**, 504 (1973).

³ Jolliffe, B. W., Rowley, W. R. C., Shotton, K. C., Wallard, A. J., and Woods, P. T., *Nature*, **251**, 46 (1974).

⁴ Evenson, K. M., *et al.*, *Phys. Rev. Lett.*, **29**, 1346 (1972).

⁵ Terrien, J., *Nouv. Revue Opt.*, **4**, 215 (1973).

Measurement of the speed of light

WE report here the completion of a determination of the speed of light at the National Physical Laboratory¹. The value was obtained from the product of the measured frequency and the wavelength, determined through up-conversion, of the radiation from a CO_2 laser stabilised to the R(12) transition of CO_2 at $9.3 \mu\text{m}$.

The frequency was determined relative to the caesium standard, using successive stages of harmonic multiplication and beat-frequency detection, with HCN and H_2O lasers used as transfer oscillators. The value obtained² was $\nu = 32,176,079,482 \text{ kHz}$, with a statistical uncertainty, σ_m , of

Accurate wavelength measurement on up-converted CO_2 laser radiation

WAVELENGTH measurements, which are part of an accurate determination of the speed of light¹, have been made on the radiation from a carbon dioxide laser, stabilised to the R(12) transition at $9.3 \mu\text{m}$ by saturated fluorescence in an external CO_2 cell, which has a measured frequency² reproducible to better than one part in 10^9 . The problems of a direct inter-comparison of infrared and visible wavelengths were avoided

by mixing the CO₂ radiation with light from a 10-mW He-Ne laser at 0.63 μm to give a difference frequency sideband at a wavelength of 0.68 μm . This mixing process, known as up-conversion, was performed in a cooled, single crystal of proustite^{3,4}. The wavelength of the CO₂ radiation was then determined using the relationship $\lambda_{0.63} = \lambda_{0.68}/(1-R)$, in which it is assumed that c is the same for the two visible radiations. The wavelength measurements were thus of the ratio $R = \lambda_{0.63}/\lambda_{0.68}$ and the value of $\lambda_{0.63}$ with respect to the primary length standard.

The value of $\lambda_{0.63}$ was determined by observing the frequency of beats against a He-Ne laser stabilised by saturated absorption of iodine, which is reproducible to better than one part in 10¹⁰. The ¹²⁷I₂ wavelength has been determined with a precision of 4 parts in 10¹⁰ by direct interferometric comparison with the ⁸⁶Kr standard⁵. Measurements from five other laboratories give a mean value within 1 part in 10⁹ of our determination⁶.

The wavelength ratio, R , was measured using Fabry-Perot interferometers with silvered plates and interchangeable fused silica spacers which incorporated piezoelectric adjustments. Two interferometric techniques were used, the first, and less precise, used a pressure scanning system developed from the arrangement used previously for precision measurements of wavelength⁵. For this first set of measurements the 10-mW laser was stabilised by reference to a Lamb dip laser which was ultimately referred by beats to component 'h' of the ¹²⁷I₂ stabilised laser ($\lambda = 632,991,369.6$ fm). Using a spacer of 187 mm we took 37 measurements. A further 11 were taken with a 15 mm spacer. These observations lead to a value for $\lambda_{0.63}$ of 9,317,246.33 pm, with an uncertainty of ± 0.06 pm ($\pm 7 \times 10^{-9}$), which is the linear sum of random and systematic uncertainties (Table 1).

Table 1 Uncertainties of pressure scanning measurement (parts in 10⁹)

Observational statistics	
Determination of $\lambda_{0.633} (^{127}\text{I}_2)$	σ_m
Determination of $(1-R)$	0.4
Short etalon	2.8
Long etalon	3.1
Resultant σ	4.2
Estimated systematic uncertainties	
Drift of Lamb dip laser (± 1 MHz)	± 2
Refractive index calculations	
Pressure (± 0.1 torr)	± 1
Temperature (± 20 mK)	± 0.5
Nonuniformity of illumination	± 1
Chromatic aberration	± 0.7
Quadrature summation	± 2.6

In the second, more precise, interferometric method the Fabry-Perot enclosure was evacuated. The interferometer was used with servo control of its length so as to be at a maximum of an interference fringe for the standard laser radiation, which was component 'd' ($\lambda = 632,991,178.3$ fm) of the ¹²⁷I₂ stabilised laser. Using electronic feedback control to the 10-mW laser, the wavelength of the up-converted radiation was adjusted so that its interference pattern was also at the maximum of a fringe. The beat frequency between the 10-mW laser and the ¹²⁷I₂ standard was counted directly. This measurement replaces the fringe interpolation necessary with the pressure scanning system. Thirty observations of the beat frequency, each of duration 10 s, were combined to give a single measurement. Using an etalon 187 mm long, 30 measurements were taken. A further 11 were taken using an etalon 19 mm long. Combined, they give a value

$$\lambda_{0.63} = 9,317,246.348 \pm 0.024 \text{ pm.}$$

The uncertainty, corresponding to $\pm 2.5 \times 10^{-9}$, is the linear sum of random and systematic uncertainties (Table 2).

Table 2 Uncertainties of servolock measurement (parts in 10⁹)

Observational statistics	
Determination of $\lambda_{0.633} (^{127}\text{I}_2)$	σ_m
Determination of $(1-R)$	0.4
Short etalon	0.84
Long etalon	0.66
Resultant σ	1.14
Estimated systematic uncertainties	
Drift of etalon length	
Short etalon	± 0.6
Long etalon	± 0.3
Nonuniformity of illumination	± 0.7
Chromatic aberration	± 0.7
Modulation of ¹²⁷ I ₂ intensity	± 0.7
Reproducibility of ¹²⁷ I ₂ laser	± 0.2
Quadrature summation	± 1.4

Differences of interferometer length, resulting from defects of flatness and parallelism, coupled with differences in the uniformity of illumination from the two sources, are important causes of error in the intercomparison of wavelengths. We measured the effect in our instrument by passing light from the 10-mW laser directly through the optical elements and diffuser normally used for the up-converted radiation. Operation of the servosystem should then result ideally in a zero beat frequency. The actual beat offset was determined using bidirectional counting, and verified as mainly systematic (7 parts in 10⁹) by interchanging the optical elements with those used for the standard. The uncertainty ascribed to modulation of the standard laser results from a possible discrepancy between the time averaged frequency and time averaged wavelength when third derivative locking⁵ is used.

The value determined with the servolock system is regarded as the final result of these measurements. The pressure scanning result was significantly less precise, although in excellent agreement. The wavelength standard throughout was the radiation of the ¹²⁷I₂ stabilised He-Ne laser. The wavelength values assigned to it are those measured here recently⁵ and in accordance with the advice of the Comité Consultatif pour la Définition du Mètre⁶, relate to the 'as maintained' metre at the National Physical Laboratory. As mentioned already, that wavelength measurement, which used the ⁸⁶Kr source, is in excellent agreement with those of other laboratories, so that the results reported here are in direct accordance with the metre as it is internationally realised.

B. W. JOLLIFFE
W. R. C. ROWLEY
K. C. SHOTTON
A. J. WALLARD
P. T. WOODS

National Physical Laboratory,
Teddington, Middlesex, UK

Received May 17; revised July 2, 1974.

¹ Blaney, T. G., *et al.*, *Nature*, **251**, 46 (1974).

² Blaney, T. G., *et al.*, *Nature*, **244**, 504 (1973).

³ Bradley, C. C., Edwards, G. J., Knight, D. J. E., Rowley, W. R. C., and Woods, P. T., *Phys. Bull.*, **23**, 15 (1972).

⁴ Baird, K. M., Riccius, H. D., and Siemsen, J. K., *Opt. Commun.*, **6**, 91 (1972).

⁵ Rowley, W. R. C., and Wallard, A. J., *J. Phys., E.*, **6**, 647, (1973).

⁶ Terrien, J., *Nouv. Revue Opt.*, **4**, 215 (1973).

A burner for mixtures of very low heat content

We must anticipate a growing demand in the future for the means to burn low grade fuels, and fuel/air mixtures which may not even be normally flammable. Upcast gases

from coal seams, ventilation air from mines, exhaust gases from a wide variety of industrial processes, and lean methane/air mixtures from some fermenting wastes are examples.

When gases such as these are allowed to escape unburnt, some particularly undesirable pollutants are added to the atmosphere quite apart from the wastage of energy. The amounts are far from negligible: it has been calculated that the methane content of the ventilation air from mines would be sufficient¹ to supply alone the entire power requirements of colliery machinery in the UK.

The majority of our combustion systems, being mixing-controlled, are quite unnecessarily inefficient, polluting and archaic^{2,3}. Even for premixed reactants, combustion theory has treated flame properties such as temperature in the reaction zone and thus burning velocity, flammability, and so on, in terms of the initial fuel/air ratio alone. Yet merely arranging for a variable amount of heat recirculation breaks this dependence⁴. Our present concern is the burning of very lean mixtures, and it has been shown⁵ that the percentage of fuel required to render a mixture flammable, decreases almost linearly with initial temperature, for example, at -0.0045% per $^{\circ}\text{C}$ for CH_4 . A study of the rate of burning of exceedingly lean methane/air mixtures, heated by an external source, has shown⁶ that preheating gases in a furnace to, for example, $1,000^{\circ}\text{C}$, would ensure the complete reaction of even 0.5% CH_4 in air within 10^{-3} to 10^{-2} s.

Providing this heating by the 'borrowing' of some of the product enthalpy, without simultaneous dilution of reactants by products, has been considered⁴, and the beneficial effects on limiting burning rates and compositions, on pollutant emission, on fuel saving and on the efficiency of conversion to electrical power when the unconverted energy is incorporated in the heat recycle, have been calculated⁷. Although the feasibility of such a scheme was demonstrated experimentally, this was carried out using a counter current heat exchanger surrounded by a Dewar flask, all constructed in fused silica⁷ which was too trans-

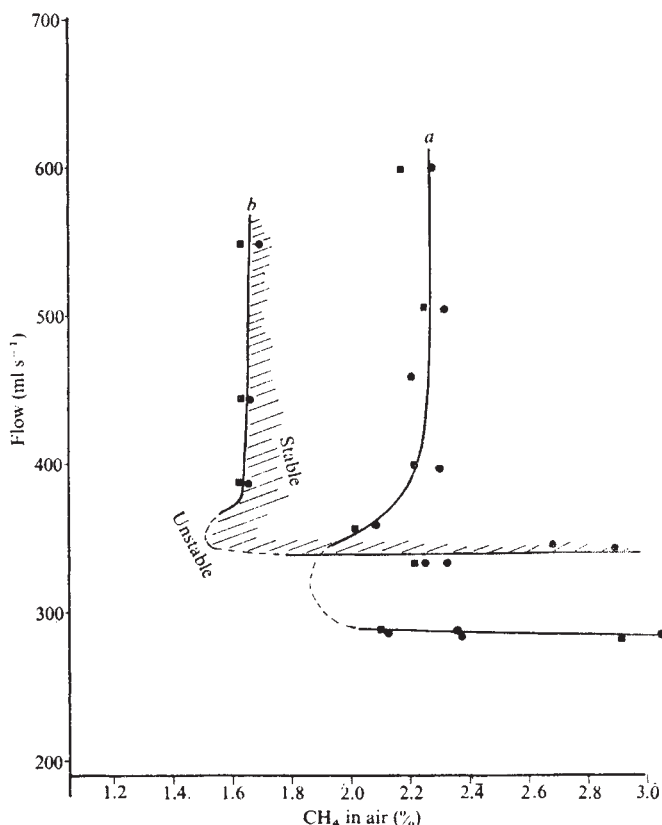


Fig. 2 Steady state stability characteristics of two burners as a function of mixture strength. Both burners are constructed of Inconel 600 strip 5 cm wide and the flow channel dimensions are 0.4 cm by 3.5 cm. *a*, 145 cm strip made into spiral of 4.5 turns; *b*, 229 cm strip made into spiral of 8 turns. ●, Stable flame; ■, unstable flame.

NOTE: The normal limit of flammability of methane is 5.3%.

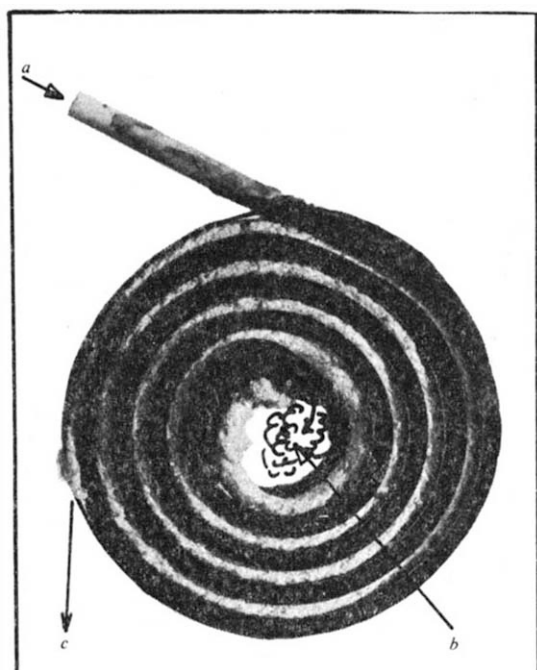


Fig. 1 Model of a burner of the type described in the text. *a*, Reactions (dark in simulation); *b*, combustion chamber; *c*, products (light in simulation).

parent in the infrared and somewhat removed from current practice. Calculations reveal that it is necessary to provide many surfaces between the combustion region and the ambient atmosphere, and the maximum possible heat exchanger area must be arranged so that a layer of cold reactants shields the device from its surroundings.

Figure 1 shows a model of a slice of virtually the simplest device which combines these attributes. The cylindrical geometry of the burner is a compromise between the theoretical ideal of spherical symmetry on the one hand and reasonable ease of construction on the other. The experimental burners were readily constructed of strips of high temperature metal alloys (Inconel 600, Nimonic 75) by winding the spirals on spacers situated at the flat ends, which were subsequently sealed with high temperature cement. For laboratory feasibility studies, relatively thin slices (less than 5 cm open channel width) were deemed adequate. Thermocouples, igniters and any other instrumentation could be inserted before sealing instead of taking the leads out in between the spiral layers. Thermocouples at the inlet and outlet of the combustion chamber allow the position of the flame to be monitored, and indicate the amount of preheat and the heat release by combustion. A thermocouple at the exit allows the assessment of total heat losses when the exit enthalpy is compared with the chemical energy fed in. Certain ceramic materials also lend themselves to this form of construction, and would be suitable if very much higher temperatures are required.

Preliminary results were obtained with combustion chambers consisting simply of a cavity at the centre of the spiral. The reactants enter it through apertures in the cen-

tral fold of the metal strip. As with any other type of burner, the flame is stable for a limited range of flow rates; one difference which arises, however, is that blow-off, consequent on too high a flow, or flash-back, which occurs when the flow is too low (that is equivalent to premature ignition) decreases the effective length of the heat exchanger by moving the flame away from the centre of the spiral. This introduces an automatic self stabilising mechanism against flash-back. Figure 2 shows two limiting stability curves (flow velocity against fuel/air ratio) illustrating the effects of varying the length of the strip and of the geometry of the combustion chamber. Even with the present small sizes (see Fig. 2) extended ranges of stable burning were obtained for mixtures of fuel content corresponding to 1/3 the normal limit of flammability, and a much smaller fraction of the leanest mixtures stabilisable on conventional burners. At this point the amount of heat recirculated is more than double that released by the reaction.

The potentialities of such devices range from burning up undesirable pollutants, through association with fluidised beds, to direct power generation. The design in which a high temperature fluidised bed combustor contained in a thoria crucible is located at the centre of the spiral, permits heat removal at the maximum temperature either by radiation or by a heat pipe. Association with Stirling motors seems promising. Here again the ability to vary the bed temperature independently of the fuel/air ratio and the flow velocity, by varying the amount of heat recirculated, makes it possible to maintain the most desirable conditions within the combustor. Thus, poor solid fuels could be burnt at the same time as lean gas mixtures are combusted, and temperatures can be maintained below values at which concentrations of NO become appreciable.

No less interesting are the possibilities regarding the direct generation of electricity. In the past, magnetohydrodynamic, caloelectric, thermionic and other schemes have been considered in the field of combustion⁸, but with little success. Several attributes of the present burners make it seem worth while to reconsider some of these schemes. One such attribute is the automatic feedback of the unconverted heat to the incoming reactants. Another is the convenient geometry of the burner (for example for the application of magnetic fields) and the very large area of metal which it maintains at a high temperature with the smallest possible input of fuel. In the small experimental devices under test, the ratio of the surface area of the metal strip to the cross-sectional area of the duct is over 2,000 and a throughput of about 200 W maintains approximately 0.1 m² of metal at white heat. There can be little doubt that a thermionic generator constructed in a shape suitable for this spiral burner would yield an excellent overall efficiency⁷ with lean mixtures.

We carried out a simple experiment at atmospheric pressure, based on the supposition that, as the paths which the charge carriers have to cross is only of the order of 4 mm, many electrons will remain unattached⁸. We coated two small electrode strips, one with barium oxide (to emit electrons) and the opposite one with potassium chromate (the positive ions from which would help to neutralise their space charge). The strips were again separated with insulating spacers, surrounded by radiation shields to simulate the coils of the spiral, and heated to various temperatures by flame gases while the currents flowing between them, and the potential, were measured. The current gradually decayed with time and there was an associated deposition (presumably from the potassium chromate) on the barium oxide. Potentials were of the order of 0.6–0.8 V, and graphs of log (current density) against $1/T$ gave straight lines corresponding to an activation energy of 1.81×10^5 J mol⁻¹. Maximum current densities extrapolated to zero time and a temperature of 1,200°C were about 200 A m⁻². These current densities are of course orders of

magnitude smaller than the least values that have, in the past, been considered necessary to render viable an evacuated diode thermionic generator. Our objective was not to produce a generator but merely to illustrate the feasibility of the principle; no doubt many other coatings and systems would yield better results. Even so, at 1,200°C the maximum initial power density of about 150 W m⁻² could yield an output of several tens of watts if an appreciable area of our small experimental spiral were maintained at that temperature. This is to be compared with an input enthalpy flow rate of 200 W already attained in our preliminary experiments. When used in this capacity, the spiral would, in any case, be built up to a large diameter so that the exit temperature, after abstraction of electrical power, need not exceed that of the incoming gas by more than the very small amount required to drive the heat transfer.

We therefore conclude that devices of this kind are not only capable of burning exceedingly lean fuel/air mixtures—well below the conventional flammability limit—but may, with some experimental refinements, also lend themselves to direct power generation from such mixtures.

S. A. LLOYD
F. J. WEINBERG

Imperial College,
London SW7, UK

Received May 13; revised June 11, 1974.

- ¹ Roxbee Cox, H. *Proc. Instr. mech Engrs*, **164**, 407–424 (1951).
- ² Weinberg, F. J., *Proc. R. Instn Gt Br.*, **45**, 299–333 (1972).
- ³ Weinberg, F. J., *Bull. Inst. Phys., London*, **25**, 56 (1974).
- ⁴ Weinberg, F. J., *Nature*, **233**, 239–241 (1971).
- ⁵ Smith, I. E., thesis, Univ. London (1955).
- ⁶ Burgoyne, J. H., and Hirsch, H., *Proc. R. Soc.*, **A227**, 73–93 (1954).
- ⁷ Hardesty, D. R., and Weinberg, F. J., *Combust. Sci. Technol.*, **8**, 201 (1974).
- ⁸ Lawton, J., and Weinberg, F. J., *Electrical Aspects of Combustion* (Clarendon Press, Oxford, 1969).

The stability of connected linear systems

GARDNER and Ashby¹ have given an account of the sudden onset of instability at a particular connectance in a set of N first-order linear equations, described by $\dot{x}_i = A_{ij} x_j$. They gave graphs for $N = 4, 7$ and 10, showing how the probability of stability varied with the connectivity, C , which was defined as the fraction of the coefficients A_{ij} ($i \neq j$) allowed to be non-zero. These couplings, A_{ij} , were uniformly distributed randomly between +1.0 and -1.0, and the diagonal terms, A_{ii} , were uniformly distributed between -0.1 and -1.0. This result seemed to be of great importance as a model for many real systems.

As a preliminary to a study of the effects which it has been anticipated would occur if there were strongly local structure, we have repeated these calculations, but could not confirm the sharp, stepwise onset of instability. Figure 1 shows our results compared with those of Gardner and Ashby¹.

Points were calculated by actually finding the eigenvalues and examining them for real parts equal to, or greater than, zero (using the Nottingham Algorithms Group subroutine for non-symmetrical real matrices). Figure 2 shows the low-connectivity region on an increased scale.

R. M. May³ examined the situation theoretically and concluded that the critical connectivity, C , is given, for sufficiently large N , by $a^2 NC = 1$, where a^2 is the mean square value of the non-zero A_{ij} . He finds that "the relative

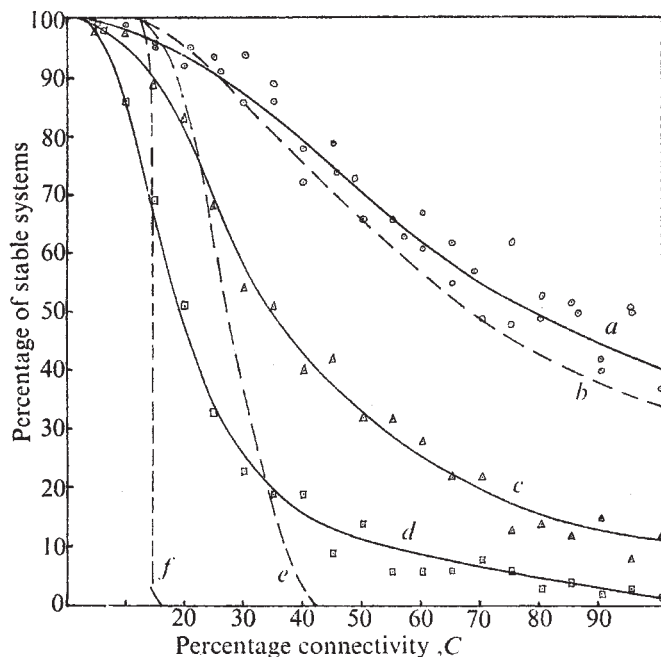


Fig. 1 Connectivity, C , against percentage of stable systems. Each point corresponds to 100 randomly filled matrices, except in cases of $N=20$. Dotted lines, results of Gardner and Ashby¹; solid lines, our results. a , $N=4$; b , $N=4$; c , $N=7$; d , $N=10$; e , $N=7$; f , $N=10$.

width of the transition region, scales as $N^{-2/3}$.

From the slopes of the curves where they are roughly linear, we found that the width of the transition region (the inverse of the slope) is approximately proportional to $N^{-2/3}$. Ashby and Gardner¹ found a much steeper function; the width approached zero for $N=10$.

It seems that, given a number of systems which are stable in isolation from one another, increasing the connectivity (keeping equal probabilities of positive and negative couplings) simply increases the probability of instability. All authors are agreed upon that general conclusion. The conjecture of Gardner and Ashby, that the sudden change from stability to instability occurs at a certain critical non-zero connectivity, has not been confirmed. Therefore, although earlier comments²⁻⁴ on that particular step-like feature remain valid in their general analyses, they can be refuted once it has been shown, as it has been shown here, that the feature is an artefact of the computations of Gardner and Ashby¹.

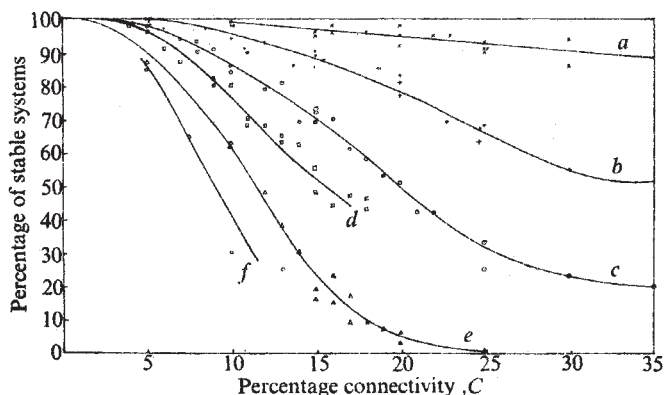


Fig. 2 Connectivity, C , against percentage of stable systems for the low-connectivity region on an increased scale. Each point represents 100 repetitions except for $N=20$. a , $N=4$; b , $N=7$; c , $N=10$; d , $N=12$; e , $N=16$; f , $N=20$.

Computer experiments on the effects of localising the connectivity are continuing.

JUDITH DANIELS

Computer Centre,
University College London,
19 Gordon Street,
London WC1H 0AH

A. L. MACKAY

Department of Crystallography,
Birbeck College,
(University of London),
Malet Street,
London WC1E 7HX, UK

Received May 31, 1974.

¹ Gardner, M. R., and Ashby, W. R., *Nature*, **228**, 784 (1970).

² Siljak, D. D., *Nature*, **249**, 280 (1974).

³ May, R. M., *Nature*, **238**, 413-414 (1972).

⁴ Somorjai, R. L., and Goswami, D. N., *Nature*, **236**, 466 (1972).

Evolution of organic compounds in volcanic regions

ACCORDING to Oparin¹, the origin of life is a natural consequence of the long evolution of organic compounds. Much effort has been devoted to the abiogenic synthesis of biologically important compounds² but because little is known about conditions on the primitive Earth, the results of such experiments are inconclusive. For this reason, the examination of volcanoes, hydrothermal systems and submarine volcanoes as sources of organic compounds seems to be highly promising.

In fact, several theoretical^{3,4} and experimental studies⁵ have confirmed the thermal abiogenic synthesis of a wide range of organic compounds, and it may be possible to confirm the formation of organic compounds in volcanic regions.

Amino acids and nucleic acid bases, such as adenine⁶, can be easily synthesised in the presence of hydrogen cyanide, so the discovery of cyanide and thiocyanate compounds in areas of volcanic activity is essential if the volcanic model of the evolution of organic compounds is to be substantiated. The results of examining volcanic material for the presence of some prebiological components (hydrogen cyanide and its derivatives) are given below. In the search for these compounds some areas in Kamchatka and the Kuriles Islands are very suitable because intensive outbursts of juvenile material are observed in many eruptions there.

From June to July 1973, the volcanic areas of the Kuriles (the Mendelev and Golovnin volcanoes on Kunashir Island; the Alaid volcanoes on Atlasov Island) and Kamchatka (the Uzon, Malyi Semyachek and Mutnovsky volcanoes) were explored.

Some thermal springs in the caldera of the Golovnin volcano (Kunashir Island) contain thiocyanates and hydrogen sulphide. This indicates the primary formation of hydrogen cyanide with its subsequent transformation into thiocyanates. Thiocyanates have been recorded previously⁷ in other volcanic areas.

In the water of several thermal springs in the caldera of the Uzon volcano (Kamchatka), cyanides were detected in the form of soluble ferrocyanides confirming some previous data⁸. As might be expected, free hydrocyanic acid has not been found in these springs, because it is very reactive and because a large number of accompanying substances, such as sulphur in various stages of oxidation, are present.

Free hydrocyanic acid can exist in active volcanic regions in conditions of fairly high temperatures and in the complete absence of substances that interact with it. Such conditions are found in the lava stream area on the Alaid volcano (the Kuriles) that erupted in 1972 and hydrocyanic acid was discovered in concentrations of the order of 0.01 mg l^{-1} in gas samples with a temperature of about 900°C that were taken from crevices in sheets of lava. The presence of the acid was determined by converting hydrocyanic acid to cyanogen chloride later identified using glutacetaldehyde (which forms a polymethine dye with 5,5-dimethyl-1,3-cyclohexane dionin) and also by the Berlin blue test. The presence of hydrocyanic acid derivatives in volcanic exhalations has been noted before, but the detection of hydrocyanic acid proper calls for some additional discussion.

The character of the hydrogen cyanide detected is very important because it can also result, for instance, from a pyrolytic destruction of nitrogen-containing organic compounds. Nevertheless, the sampling conditions and the geological structure of the slag cone of the Olimpiiskii break (the site where gas samples were taken) in the Alaid volcano, point to the 'juvenile' character of recorded hydrocyanic acid.

The analysis of the volcanic gases and the temperatures indicate that hydrocyanic acid is a probable component of volcanic activity. The experimental data obtained can be considered as a confirmation of an earlier hypothesis about the role of volcanism (particularly submarine volcanism) and thermal springs in the processes of prebiological evolution on the primitive Earth.

Indeed, in strong eruptions, approximately 10^9 cubic metres of gas are released. If my results are correct, the amount of hydrogen cyanide liberated into the atmosphere in a single eruption would be about 10^7 g. Evidently volcanism must have played a significant role in the synthesis of organic compounds. Amino acids have also been detected in several thermal springs. But conclusions about the 'juvenile' nature of these compounds can be made only after undertaking additional investigations.

LEV MUKHIN

Space Research Institute,
USSR Academy of Sciences,
USSR

Received March 28, 1974.

- ¹ Oparin, A. I., *The Origin of Life on Earth* (AN CCCP, Moscow, 1957).
- ² Ponnamperna, C., and Gabel, N. W., *Space Life Sci.*, **1**, 64-96 (1968).
- ³ Mukhin, L., *Communication with Extra-Terrestrial Intelligence*, Symposium at Byurakan (edit. by Sagan, C.), 58 (1972).
- ⁴ Fox, S. W., *The Origin of Prebiological Systems* (Academic Press, New York, 1965).
- ⁵ Rittmann, A., *Vulkane und Ihre Tätigkeit*, Stuttgart, Aufl., 1936, Aufl. (1960).
- ⁶ Pavlov, A. L., Karpov, G. A., *Dokl. AN CCCP*, No. 3, **206**, 719 (1972).

experimental production of diabetes mellitus in animals by induction of slow consumptive fibrinocoagulopathy.

A batch of 14 rabbits, weighing 1.5-2 kg was studied. The animals were kept on a standard pellet diet of the following composition; crude protein, minimum 20.0%; ether extract, minimum 6.0%; crude fibrin, maximum 4.0%; ash maximum 8.0%; calcium, minimum 1.5%; phosphorus, minimum 0.6%; nitrogen-free extract 48.0%; metabolisable energy, 3,000 calorie kg^{-1} . Mineral stabilised vitamin A and D_3 (Roche), vitamin E, vitamin K, vitamin B_{12} , vitamin C, thiamine, riboflavin, pantothenic acid, niacin, choline chloride, folic acid and trace minerals (cobalt, copper, iron and manganese) were also added to this formulation. The animals were fed with 60 g of the pellet diet and 110 g of vegetables and clean fresh water daily.

The production of 'slow consumptive fibrinocoagulopathy' was achieved by intermittent administration of intravenous bovine thrombin (Upjohn, USA) in small doses. The optimum dose of thrombin was determined by trying graduated doses of bovine thrombin from 5 National Institute of Health (NIH) units to 13 NIH units.

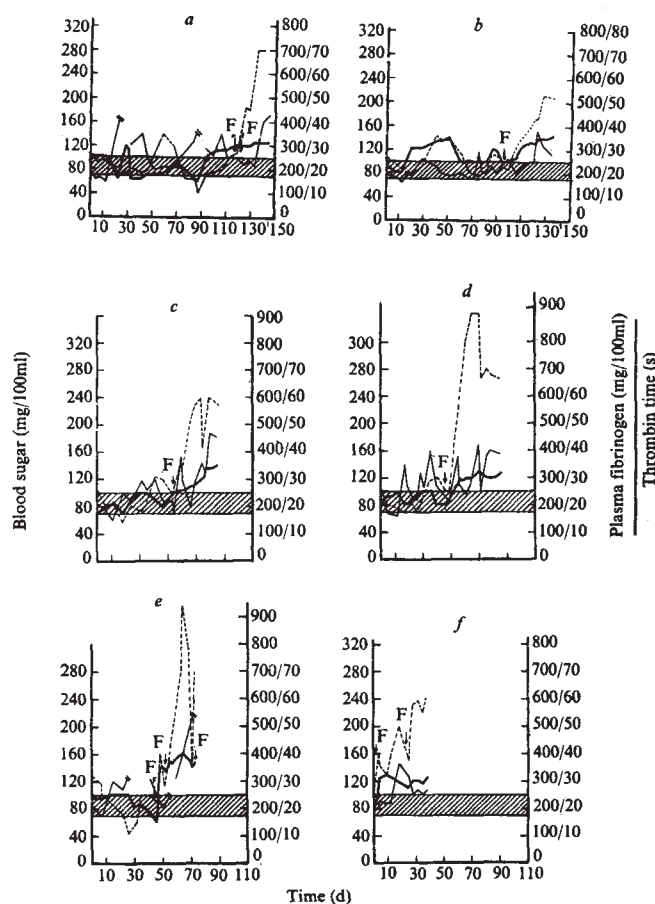


Fig. 1 Blood sugar (—), plasma fibrinogen (— — —) and thrombin time (—) in six fibrinopathic diabetic animals. Shaded area shows the normal range (70-100 mg per 100 ml) of blood sugar. F, intravenous injections of bovine fibrinogen. a, Rabbit No. 2; b, rabbit No. 5; c, rabbit No. 8; d, rabbit No. 10; e, rabbit No. 11; f, rabbit No. 12.

While no significant alterations were observed with 5 NIH units, rapid deaths occurred with 12 and 13 NIH units. The thrombin dose was accordingly standardised at 10 NIH units, diluted in 10 ml normal saline, and administered at the rate of $1 \text{ U ml}^{-1} \text{ min}^{-1}$. A faster rate of injection and higher concentrations of the thrombin solution produced fatal results. On this dosage schedule, it was possible to keep the animals alive in a state of fibrinocoagulopathy. The thrombin solution was routinely injected through the ear vein of the animals.

The haematological assessment for the fibrinocoagulopathic

Experimental production of diabetes mellitus by a nonendocrinal approach

THE immunofluorescent demonstration of large deposits of fibrin in the microvascular lesions^{1,2}, and evidence of a slow consumptive fibrinocoagulopathy in maturity onset diabetes mellitus^{3,4}, suggested the possibility of a primary fibrinocoagulopathic vascular aetiopathogenesis of the disorder. The present work was undertaken to investigate the possibility of an

Table 1 Results of experimental fibrinocoagulopathy in 14 animals

Rabbit No.	Dose of thrombin administered	Rate of thrombin infusion	Haematological state	Clinical result
1 Standardisation of thrombin dose and rate of infusion				
R ₁	12 NIH/ 5 ml	1 ml min ⁻¹	—	— Immediate death
R ₃	13 NIH/10 ml	1 ml min ⁻¹	—	— Immediate death
R ₆	10 NIH/10 ml	Rapid injection (5 U min ⁻¹)	—	— Immediate death
R ₉	10 NIH/10 ml	Rapid injection (2 U min ⁻¹)	—	— Immediate death
2 Non fatal vascular complications				
R ₇	10 NIH/10 ml	1 ml min ⁻¹	Fibrinocoagulopathic state with prolonged thrombin time and reduced plasma fibrinogen (< 350 mg/100 ml)	Developed sudden hind limb paralysis after eleven injections without developing hyperglycaemia. Experiment discontinued (blood sugar < 100 mg/100 ml)
3 Experimental fibrinopathic diabetes mellitus				
R ₂	10 NIH/10 ml	1 ml min ⁻¹	Maintained 'Slow fibrinocoagulopathic state', with prolonged thrombin time and plasma fibrinogen above 350 mg 100 ml	Sustained diabetes mellitus (blood sugar 110–160 mg/100 ml)
R ₅				
R ₈				
R ₁₀				
R ₁₁				
R ₁₂				
4 Central group				
R ₄	No thrombin injection; normal saline 10 ml injected intravenously		Normal thrombin time 20–22 min with 10 NIH units of thrombin and plasma fibrinogen 270–320 mg per 100 ml	Euglycaemic (Blood sugar 70–100 mg per 100 ml. Mean 87.7 mg per 100 ml)
R ₁₃				
R ₁₄				

state was done by carrying out the thrombin time estimation by the method as described in our earlier communications³⁻⁵, and plasma fibrinogen estimation as described by Ratnoff and Menzie⁶.

After the initial induction of fibrinocoagulopathy, hypofibrinogenaemia was prevented in the animals by maintaining the plasma fibrinogen level above 350 mg per 100 ml, while on thrombin treatment, by periodic intravenous administration of 50 mg of bovine fibrinogen dissolved in 10 ml normal saline.

The blood sugar estimation was carried out by the method of Asatoor and King⁷. A batch of three central animals were kept on the standard diet schedule and received injections of the diluent normal saline used in the experiment. A 'double blind' method with the coded blood samples, by two independent teams of laboratory workers, was adopted in carrying out the

plasma estimations. Results of the study of 14 animals are shown in Table 1 and in Figs 1 and 2.

Our experimental procedure successfully developed a state of slow consumptive fibrinocoagulopathy in seven animals (R₂, R₅, R₇, R₈, R₁₀, R₁₁, R₁₂) and these were maintained in that state to study the alterations in the blood sugar level.

In the three control animals (R₄, R₁₃, R₁₄), on scheduled diet regime, the blood sugar values ranged from 70 to 100 mg per 100 ml, with a mean value of 87.7 mg per 100 ml and the animals remained euglycaemic for the entire experimental period of 6 months, without alterations in the thrombin time or plasma fibrinogen level.

In the experimental group, all seven animals showed an elevation in blood sugar values with the induction of consumptive fibrinocoagulopathy, in about 2–4 weeks' time. The primary rise in the blood sugar levels, however, showed a decline with the hypocoagulability and reductions in the plasma fibrinogen concentrations. In animal R₇, the primary elevation was within normal range, while the remaining six animals (R₂, R₅, R₈, R₁₀, R₁₁, R₁₂) became hyperglycaemic with values above 110 mg 100 ml.

In all six animals (R₂, R₅, R₈, R₁₀, R₁₁, R₁₂) (100%), the hyperglycaemia could be reinduced by raising the plasma fibrinogen level above 350 mg per 100 ml, by supplementary injections of 50 mg of bovine fibrinogen. Thereafter the animals remained diabetic, being maintained on fibrinocoagulopathic state with the plasma fibrinogen level above 350 mg per 100 ml (Fig. 1).

Animal R₇, however, was not given any supplementary fibrinogen injection and the animal continued to be euglycaemic in a state of consumptive fibrinocoagulopathy till it developed acute spastic hind limb paralysis indicating a macrovascular occlusive lesion of the spinal artery (Fig. 2).

In the case of R₁₂, the animal was given the supplementary dose of fibrinogen on the first day along with the primary dose of thrombin and developed a sustained diabetic state right from the very beginning, without showing any decline in the blood sugar values (Fig. 1, R₁₂).

The observations therefore provide convincing experimental evidence of a production of diabetic state by consumptive fibrinocoagulopathy in the animals. The critical factor in developing the fibrinopathic hyperglycaemia seems to be a normal or raised plasma fibrinogen in a state of consumptive fibrinocoagulopathy in the animals. As far as we are aware, this is first reported since the discovery of insulin, of an experimental

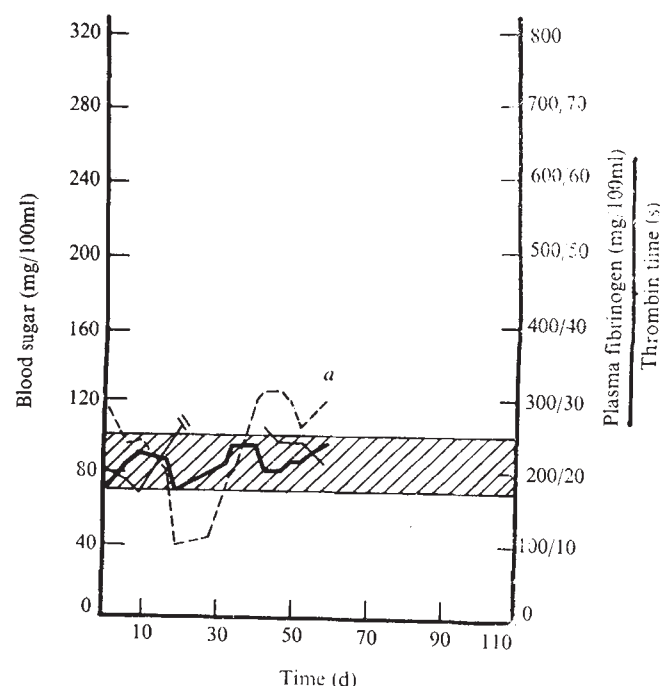


Fig. 2 Blood sugar (—), plasma fibrinogen (---) and thrombin time (—) in animal R₇. Shaded area shows the normal range of blood sugar. a, Acute spastic paraplegia.

production of diabetes mellitus by a nonendocrinal approach.

This work was carried out by grants from the Research and Development Organisation, Ministry of Defence, Government of India. We thank Brigadier S. Gopal Krishnan, Medical Superintendent, Safdarjang Hospital, for permission to carry out the experimental work. The technical co-operation and assistance of Subhash Chander and D. Das are gratefully acknowledged.

R. N. BANERJEE
S. RAMACHANDRA RAO
JAYA BARDHAN

Department of Haematology and Nuclear Medicine,
Safdarjang Hospital,
New Delhi 16, India

Received December 17, 1973; revised January 13, 1974.

- ¹ Davies, M. J., Woolf, N., and Carstairs, G., *J. Path. Bact.*, **92**, 441 (1966).
- ² Farquahar, A., Donald, M., Mark, K., and Ireland, J. T., *J. clin. Path.*, **25**, 857 (1972).
- ³ Banerjee, R. N., Sahni, A. L., and Kumar, V., *Ind. J. med. Res.*, **60**, 1432 (1972).
- ⁴ Banerjee, R. N., Sahni, A. L., and Kumar, V., *Thromb. Diathes. Haemorrh.*, **30**, 123 (1973).
- ⁵ Dacie, J. V., and Lewis, M. S., *Practical Haematology*, ELBS fourth ed., **274**, (1968).
- ⁶ Ratnoff, O. D., Menzie, C., *J. lab. clin. Med.*, **37**, 316 (1951).
- ⁷ Asatoor, and King, *Biochem. J.*, **10**, 56, (1954).

Mode of action of ancrod as a defibrinating agent

ANCROD is a thrombin-like enzyme obtained from the venom of *Agkistrodon rhodostroma*. When added to plasma, ancrod initiates fibrin polymerisation by the cleavage of fibrinopeptide A from fibrinogen¹ but activates factor XIII (plasma transglutaminase) very slowly². Factor XIII, when activated, catalyses the formation of crosslinks between the α chains and between the γ chains of fibrin³. The absence of these cross-linked chains, especially the α chain polymers, allows fibrin to be easily degraded by plasmin^{4,5}. Thus it is believed that ancrod acts as a defibrinating agent *in vivo* by producing non-cross-linked easily lysable fibrin deposits. We wish to present some preliminary evidence to show that commercial preparations of ancrod activate factor XIII in plasma *in vitro* and may also mediate the conversion of prothrombin to thrombin. In addition a hypothesis is presented to explain the defibrinating effect of ancrod *in vivo*.

Human fibrinogen (Grade L from KABI, Stockholm, Sweden) and bovine (Diagnostic Reagents, Thame, Bucks) or human (Parke-Davis) thrombin were used in these experiments. Human plasma was collected in 0.38% sodium citrate and stored in aliquots of 1 ml at -70°C when not used immediately. Hirudine, an inhibitor of thrombin activity, was obtained from Sigma, Surrey, and a naturally prothrombin deficient plasma was donated by Dr P. Barkhan (Guy's Hospital, London) through the offices of Dr M. Brozovic of this Institute. This prothrombin deficient plasma was immunologically and biologically devoid of prothrombin activity. Clinical grade ancrod (Arvin, batch F1623) and a more highly purified extract (IRC 50 Arvin, batch DRP 1/143) were donated by Mr G. W. Tunnah of Twyford Laboratories, Beckenham, Kent.

Sodium dodecyl sulphate polyacrylamide (SDS-PA) gel electrophoresis was performed by the method of Weber and Osborn⁶. This technique has been successfully used to examine the subunit composition of the degradation products of fibrinogen by plasmin⁷ and the degree of crosslinking of the γ chains and α chains of clotted fibrin³. Artificially made (BaSO₄

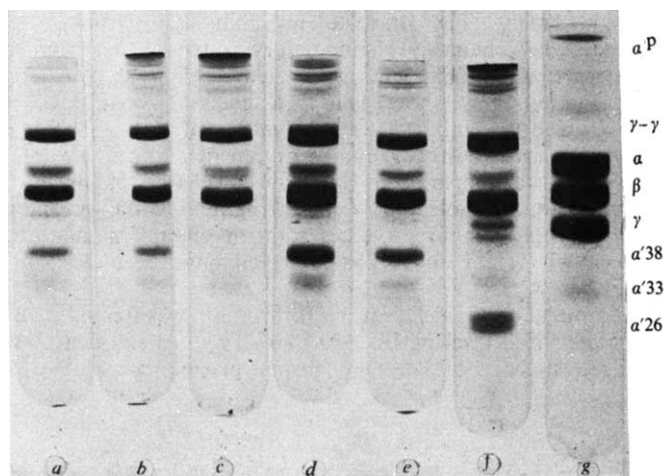


Fig. 1 Sodium dodecyl sulphate polyacrylamide (SDS-PA) electrophoresis (7% gel) of polypeptide chain subunits of fibrins obtained from plasma and from purified fibrinogen by treatment with ancrod preparations and thrombin. *a*, Plasma fibrin obtained by Arvin, batch F1623; *b*, plasma fibrin by IRC-50, batch DRP 1/143; *c*, plasma fibrin by thrombin; fibrins from purified fibrinogen by *d*, Arvin, batch F1623; *e*, IRC-50 Arvin, batch DRP 1/143; *f*, thrombin. Pattern *g*, show the three chains of fibrin α , β , γ . Electrophoresis was conducted for 16 h at 2 mA per gel. The gels were stained for 3 h with 0.25% Comma-blue in methanol/acetic acid/water (45/5/50) and destained in the same solvent. * Molecular weight $\times 10^{-3}$.

absorption) prothrombin-deficient plasma was treated separately with ancrod (40 Twyford units per ml plasma, 9 mM CaCl₂) and thrombin (2 NIH units per ml plasma, 9 mM CaCl₂) as was a 0.3% prothrombin-free fibrinogen solution (0.9% NaCl-0.4% Na citrate). After incubation at 37°C for 2 h the plasma fibrins were washed in saline-0.2 M ϵ -amino-caproic acid (ϵ -ACA) and reduced overnight in 4 M urea-1% SDS-0.2 M β -mercaptoethanol before examination by electrophoresis. The results are shown in Fig. 1. The γ chains are crosslinked and there are no α chain polymers in the Arvin-mediated plasma fibrin; however an α chain fragment of molecular weight 38,000 is evident (Fig. 1*a*)^{1,8,9}. The IRC-50 Arvin-treated plasma fibrin was made up of β chains, γ dimers, some α polymers and some α chain fragments (molecular

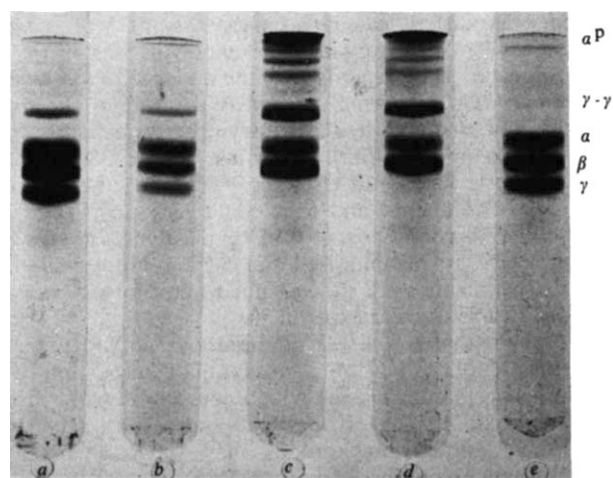


Fig. 2 Polypeptide subunits on SDS-PA gel (7%) electrophoresis of plasma fibrin obtained by IRC-50 Arvin from *a*, plasma containing no prothrombin; *b*, normal plasma with hirudine; *c*, normal plasma. Pattern *d* shows chains of fibrin obtained from plasma with thrombin; and pattern *e* shows the chains of non-crosslinked fibrin. Other details as in Fig. 1 legend.

weight 38,000) (Fig. 1b). The thrombin-fibrin from plasma contained β chains, γ chain dimers and α chain polymers but no α chain fragments (molecular weight 38,000) (Fig. 1c). Figure 1 (d-f) shows that high levels of Arvin, IRC-50 Arvin and thrombin cause activation of factor XIII to crosslink most of the γ chains of purified fibrin; also that both anicrod preparations lyse the α chains of fibrin to form a 38,000 molecular weight fragment while thrombin does not. Thrombin produces some α chain fragments of molecular weight about 26,000 which have been described elsewhere¹⁰.

Plasma naturally deficient in prothrombin was treated with a clinically used level of IRC-50 (0.07 Twyford units ml⁻¹) in the presence of 9 mM CaCl₂. Normal plasma, containing 250

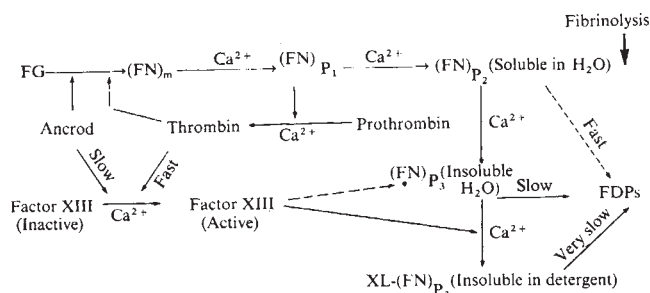


Fig. 3 Hypothetical scheme of anicrod generation of fibrin (FN) from fibrinogen (FG) *in vivo* and the ability of the fibrinolytic system to lyse the various possible fibrin polymers ($P_1 < P_2 < P_3$) to fibrin degradation products (FDPs). XL = crosslinked α chains and γ chains.

units of Hirudine ml⁻¹, was treated with IRC-50 Arvin while normal plasma samples (1 ml) treated separately with 0.07 Twyford units of IRC-50 Arvin and 0.25 NIH units of thrombin, were used as controls. The fibrins were examined for their subunits on SDS-PA electrophoresis and the results are shown in Fig. 2. It is evident that Arvin IRC-50 by itself slowly activates factor XIII (as shown by the presence of some γ chain dimers) when there is no prothrombin present (Fig. 2a) or when thrombin is totally inhibited by hirudine (Fig. 2b). It is notable that at these concentrations of anicrod no α' chain fragments (molecular weight 38,000) are produced and no α chain polymers are found during the 2 h incubation when the prothrombin-thrombin complex is absent or inhibited. In normal plasma (prothrombin present) the subunit pattern (Fig. 2c) with low level Arvin is the same as that obtained with thrombin (Fig. 2d). It can be seen that again there is no α chain fragment (α' 38) formed while α polymerisation (α^p) is nearly complete. This suggests that the enhanced crosslinking induced by Arvin in normal plasma is a result of thrombin activation and that low levels of Arvin activate factor XIII slowly and do not lyse fibrin α chains. The α' 38 fragment reported here (Fig. 1) and elsewhere^{2,8,9} is an effect *in vitro* of high levels of anicrod and thus could play no part in the susceptibility of Arvin-fibrin to lysis by plasmin *in vivo* as has been suggested⁸. It is emphasised that calcium levels (<9 mM) in these experiments were such as not to clot normal plasma during the course of these experiments.

It is evident that low levels of the above anicrod preparations generate fibrin which is more rapidly cross linked in normal plasma than in plasma which does not contain prothrombin (Fig. 2). We suggest that by a mechanism as yet unknown these anicrod preparations may generate thrombin assuming that, in plasma, only thrombin can activate factor XIII. The proteolytic attack by anicrod on the α chains of fibrin (Fig. 1) does not occur at a low anicrod concentration (Fig. 2) and it can be shown that the α chains can crosslink slowly to α polymers. We have recently described⁵, a fragment called D-dimer, which is formed during the lysis of fibrin clots and which contains the γ - γ crosslinks of the originating fibrin. This fragment has been also shown¹¹ to be released from

crosslinked clots of whole plasma. But plasma from patients undergoing defibrination treatment with Arvin does not contain any of this D-dimer fragment (our unpublished results). The idea, based on *in vitro* evidence, that some soluble fibrin monomer complexes can be crosslinked by their γ chains^{12,13} was not supported as we could not detect D-dimer in the plasmas of patients undergoing Arvin treatment. Since the deposition of fibrin and the formation of some γ - γ crosslinks take place at about the same time it seems that Arvin does not cause the deposition of fibrin *in vivo*. Instead fibrin, while still soluble, seems to be lysed by the fibrinolytic system in plasma.

The sequence of reactions outlined in Fig. 3 presents our current views on anicrod as a defibrinating agent, and other features of 'normal' fibrinolysis. Many aspects of this scheme need to be tested *in vitro* and *in vivo*. The main features of this hypothetical scheme are that fibrinolysis can most readily degrade soluble (under physiological conditions in plasma) fibrin polymers of size P_2 to fibrin degradation products (FDPs), that the 'lysability' of fibrin of an 'insoluble size' (P_3) and containing some crosslinks is decreased, and that totally crosslinked fibrin, XL(FN) P_3 , is resistant to plasmin degradation to FDPs. It is proposed that an intermediate size fibrin polymer, (FN)_{P1} might be responsible for the activation of plasma prothrombin noted in the presence of anicrod preparations (Fig. 2). The suggestion that fibrin activates prothrombin has been expressed elsewhere¹⁴, while both crude Malayan pit viper venom and a purified component of the latter can activate factor X^{15,16}. The newly generated thrombin would then be available to augment the weak factor XIII activation potential of anicrod and would also explain why *in vivo* low levels of anicrod can defibrinate plasma so rapidly. The scheme also suggests that when normal fibrinolysis is depressed there is a greater probability of *in vivo* formation of fibrin deposits which may be crosslinked. Recent work¹⁷ using the rabbit experimental model partly supports this aspect of the hypothesis. On the other hand it should be noted that Arvin, during infusion into man, has not caused¹⁸ the deleterious side effects which might be expected from the *in vivo* generation of thrombin.

P. J. GAFFNEY
M. BRASHER

National Institute for Biological Standards and Control,
Holly Hill, Hampstead,
London NW3 6RB, UK

Received February 18; revised April 25, 1974.

- Ewart, M. R., Hatton, M. W. C., Basford, J. M., and Dodgson, K.S., *Biochem. J.*, **118**, 603 (1970).
- Mattock, P., and Esnouf, M.P., *Nature new Biol.*, **223**, 277 (1971).
- McKee, P. A., Mattock, P., and Hill, R. L., *Proc. natn. Acad. Sci. U.S.A.*, **66**, 738 (1970).
- McDonagh, R. P., McDonagh, J., and Duckert, F., *Br. J. Haemat.*, **21**, 323 (1972).
- Gaffney, P. J., and Brasher, M., *Biochim. biophys. Acta*, **295**, 308 (1972).
- Weber, K., and Osborn, M., *J. biol. Chem.*, **244**, 4406 (1969).
- Gaffney, P. J., and Dobos, P., *FEBS Lett.*, **15**, 13 (1971).
- Pizzo, S. V., Schwartz, M. L., Hill, R. L., and McKee, P., *J. clin. Invest.*, **51**, 2841 (1972).
- Edgar, W., and Prentice, C. R. M., *Thromb. Res.*, **2**, 85 (1973).
- Gaffney, P. J., *Nature new Biol.*, **234**, 281 (1971).
- Gaffney, P. J., and Brasher, M., *Nature*, **244**, 361 (1973).
- Von Hugo, R., and Graeff, H., *Thromb. Diath. Haemorrh.*, **29**, 122 (1973).
- Kierulf, P., *Thromb. Res.*, **3**, 613 (1973).
- Dalin, G. R., and Lewis, J. H., *Nature*, **195**, 87 (1962).
- Nakas, L., Denson, K. W. E., and Macfarlane, R. G., *Thromb. Diath. Haemorrh.*, **12**, 355 (1964).
- Denson, K. W. E., *Toxin*, **7**, 5 (1969).
- Muller-Berghaus, G., and Mann, B., *Thromb. Res.*, **2**, 305 (1973).
- Sharp, A. A., Warren, B. A., Paxton, A. M., and Allington, M. J., *Lancet*, **i**, 493 (1968).

Differential effects of electrical stimulation on release of ^3H -noradrenaline and ^{14}C - α -aminoisobutyrate from brain slices

AMINO acids, especially γ -aminobutyric acid (GABA), glutamate, aspartate, glycine and taurine have been postulated to act as central neurotransmitters in vertebrates^{1,2}. Aside from GABA, which seems to have met the stringent criteria necessary for establishing its neurotransmitter role³⁻⁶, the evidence for their role is mainly circumstantial. Part of it is based on electrically induced release of amino acids from slices⁷, chopped tissue^{6,8,9} and synaptosomes¹⁰ derived from different portions of the central nervous system. In several of such studies, there are released not only the amino acids active as neuronal excitors (glutamate and aspartate) or inhibitors (glycine and taurine), but also amino acids with no known effects on the electrical activity of neurones such as lysine, arginine, leucine, cycloleucine, proline and glutamine^{7,8}. There is no criterion for believing that these last are transmitter substances.

We have studied the conditions under which electrical stimulation is a valid method for releasing neurotransmitters from brain slices. For this we have chosen to study simultaneously the efflux from neocortical thin slices of a known transmitter, ^3H -L-noradrenaline (NA) and a known amino acid non transmitter, ^{14}C - α -aminoisobutyrate (AIB). We repeatedly stimulated the slices electrically, with increasing intensities. This we call the 'voltage profile' approach. Both NA and AIB are actively transported into the cells by sodium-dependent processes: NA by a specific transport system into noradrenergic cells¹¹, when its concentration is low (under 10^{-6} M), and AIB into both neurones and glia¹² through a transport system that shows preferential affinity for small neutral amino acids¹³.

The release of ^3H -L-NA from unstimulated slices is similar to that reported previously for ^3H -DL-NA (ref. 14). Two different first-order components may be distinguished. A fast initial one represents the washout of extracellular NA, plus NA metabolites. The second component is much slower and is believed to represent the efflux of NA from intracellular stores. The efflux of ^{14}C -AIB follows a similar pattern, although the initial component is much less marked than that of NA, probably because of the nonmetabolisable nature of this amino acid.

After the first efflux component became negligible (that is, after 40 min) the slices were stimulated with increasing voltages (Fig. 1a). At low voltages (0.5–3 V), only ^3H -NA was released, in an amount roughly proportional to stimulus strength; at higher voltages the specificity of the response was lost, and both NA and AIB were released by electrical stimuli. Under

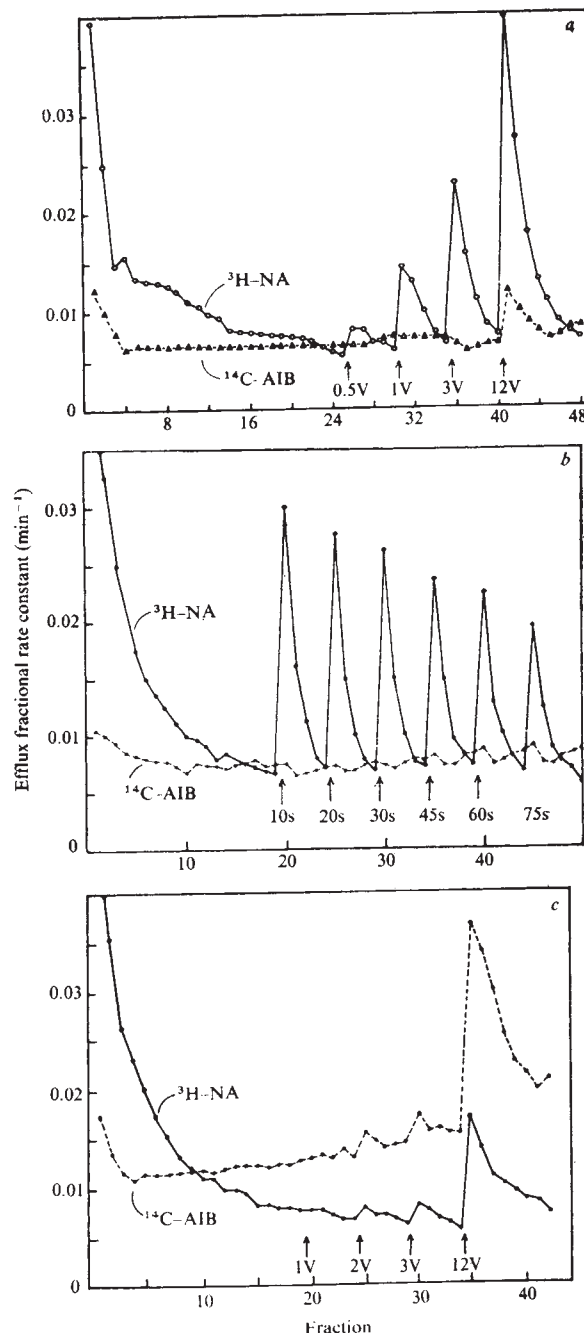


Fig. 1a Electrically induced release of NA and AIB. Slices were obtained from 120 to 150 g Sprague-Dawley rats, with blade and blade guide^{15,16}. Only the outermost slices were used. Slices of both hemispheres remained attached and could be floated together. The slices were initially incubated for 60 min in a Dubnoff shaker (37° C, 100 cycles min⁻¹) in 25 ml Erlenmeyer flasks containing 1 ml of a physiological glucose-salt solution¹⁷, equilibrated with 5% CO₂ in O₂, and 7.8×10^{-7} M ^3H -L-NA (5 μCi , specific activity 6.4 Ci mmol⁻¹) and 10^{-4} M ^{14}C -AIB (1 μCi , specific activity 10 mCi mmol⁻¹). After this period, in which both NA and AIB were actively transported into the cells, the slices were floated into a McIlwain quick transfer electrode, and this was placed in a 30 ml beaker containing 4 ml of the same salt solution, but without ^3H -NA or ^{14}C -AIB. The beaker was kept at 37° C in a thermoregulated water bath and vigorously gassed with 5% CO₂ in O₂. Every 2 min the fluid was aspirated with a 2.5 mm polyethylene tube connected at one end to a 3 mm Pyrex tube soldered to the bottom of the beaker, and at the other end to one of two perforations of a neoprene stopper fitted tightly to a 16 × 160 mm test tube. The other perforation in the stopper connected the tube, through Tygon tubing and three-way valve, to a water pump. After each aspiration the stopper was changed to a contiguous empty test tube. The beaker fluid was replenished by means of an automatic syringe that aspirated the fluid from a reservoir and injected it, through latex tubing connections, to one of the two openings of a neoprene stopper placed on another

16 × 160 mm test tube, immersed in the water bath. From this test tube, used for thermal equilibration, the fluid passed through a latex tube connected to a 3 × 80 mm Pyrex tube that was placed in the beaker. The slice was stimulated with sine wave a.c. current, 50 cycles s⁻¹, obtained from the mains line and passed through two Variac variable transformers placed in series. The stimulation voltage was monitored with a Tektronix model 532 oscilloscope, with its horizontal sweeping suppressed. The voltage then appeared as a vertical line on the screen whose height represents twice the magnitude of the maximum instantaneous voltage (MIV). All voltages are expressed as MIV. At the end of the experiment the slice was homogenised in 4 ml incubation fluid. One millilitre of each collected fraction and 0.1 to 0.2 ml of the slice homogenate (with the total volume made 1 ml with incubation fluid), were counted with 10 ml of a mixture of toluene, containing 4% PPO and 0.05% POPOP, and Triton X-100 (2:1 v/v), in a two-channel liquid scintillation spectrometer. Background subtraction, and the calculation of ^3H and ^{14}C counts in each channel were performed with an IBM digital computer. The efflux of ^3H -NA and ^{14}C -AIB were finally expressed as efflux fractional rate constants⁹. **b**, Slice stimulation with constant voltage and increasing times. **c**, Stimulation in medium with no added calcium. The initial incubation was done in normal glucose-salt solution, while the superfusion was in medium nominally without calcium. In a second experiment, identical results were found, except that no NA or AIB were released with stimuli of 0.5 V, 30 s, or 2 V, 10 s.

our experimental conditions, the limits between which electrical stimulation was a valid tool for neurotransmitter release was between 0.5 and 3 V (Fig. 2). At and above 4 V, AIB, and probably all actively transported nontransmitter amino acids, are released.

When the slices were stimulated repeatedly with 3 V for different lengths of time (Fig. 1b), a progressive decline in the induced ^3H -NA efflux, quite analogous to synaptic fatigue became apparent. With stimulation times longer than 30 s, small increases in AIB efflux could also be elicited. In stimulated slices, the efflux of AIB assumed an undulating character, not seen in the absence of stimuli, that might be related to fluctuations in the concentrations of intracellular sodium.

Markedly different results were obtained when the efflux of NA and AIB was studied in slices superfused with calcium-free medium (Fig. 1c). The efflux of ^3H -NA did not increase when the slices were stimulated with 0.5 and 1 V, and the extra efflux induced by higher potentials was greatly reduced. ^{14}C -AIB was however now released by stimuli as weak as 2 V, and at 12 V, its stimulated efflux is considerably larger than when calcium was present. Independently of the stimulation voltage, the efflux of AIB also now showed a steady increase with time.

The most straightforward interpretation of the electrically

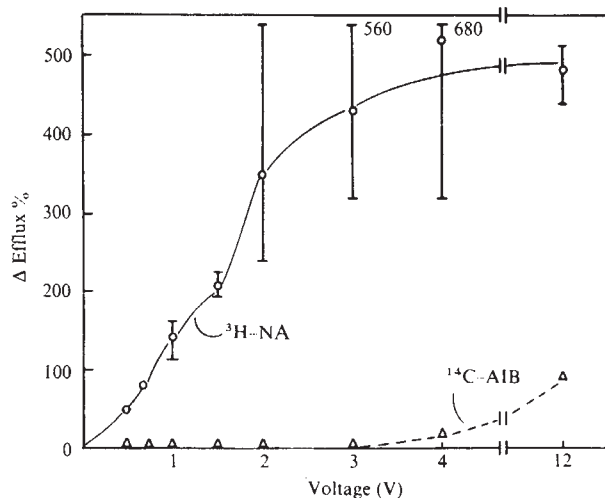


Fig. 2 Differential effects of electrical stimulation on NA and AIB release. Each point represents 3–5 estimations. Ranges are shown as vertical lines. All the triangles in the 0 to 3 V region have zero values.

induced release of AIB is based on the sodium hypothesis¹⁸ of active amino acid transport. Release seen at high stimulation potentials is probably due to increased intracellular sodium¹⁹ that enhances the affinity for AIB of the transport carrier on the inside of the membrane. Similarly, it is known that in calcium-deficient media, sodium influx during stimulation is increased²⁰. This should also lead to an enhanced amino acid efflux during stimulation. Finally, independently of stimulation, brain slices placed in media nominally without calcium²¹ steadily increase their intracellular sodium¹⁷. This correlates well with the steady increase in AIB efflux seen in calcium-free medium.

It seems likely that the electrically induced release of NA seen at low stimulating potentials is not immediately dependent on the reverse operation of a sodium activated, carrier mediated, transport system, but represents true calcium-dependent exocytotic neurotransmitter secretion. With more intense stimuli, both calcium-dependent and sodium-dependent mechanisms may be set in operation. The small electrically induced NA release seen in medium that lacks calcium could be interpreted as being due to the presence of residual amounts of calcium which still support some secretion. But the strict parallel that exists under these conditions between the electrically induced release of NA and of AIB, strongly suggests

that all of the NA released in the absence of calcium is due to the reverse operation of the sodium-activated NA membrane transport system. The source of NA is presumably free NA present in the cytoplasm. The coexistence of sodium-dependent and calcium-dependent neurotransmitter release processes, in these *in vitro* preparations, with a great enhancement of the sodium processes when no calcium is present, makes it very difficult to demonstrate an absolute calcium requirement for neurotransmitter release. For this, stimulation conditions must be very carefully controlled, and there should be internal controls for sodium-dependent processes, as is the case with AIB efflux.

The voltage profile approach is essentially a qualitative procedure, by which substances released by electrical stimulation may be classified as neurotransmitter-like or amino acid-like; the actual amount of substance released is, in itself, irrelevant for this classification. This approach may be applied to any of the various systems used for superfusing and stimulating nerve tissue preparations *in vitro*, although the stimulation parameters will vary considerably from one system to another. Its use will determine whether many of the substances reported to be released by electrical stimulation behave in a transmitter-like or in an amino acid-like manner. This approach may however only be used with stimuli of alternating polarity, because monophasic pulses rapidly polarise the electrodes.

We are grateful to Mr P. Frigerio for constructing the quick transfer electrodes, to O. Petit for technical assistance, to E. Moraga for the computer program and to Conicyt and Comisión de Investigación Científica, Universidad de Chile, for financial help.

F. ORREGO
J. JANKELEVICH
LEONOR CERUTI
E. FERRERA

Instituto Nacional de Cardiología,
Av. Cuauhtémoc 300,
México 7, DF
and Departamento de Fisiología y Biofísica,
Universidad de Chile,
Santiago, Chile

Received March 4; revised April 5, 1974.

- Curtis, D. R., and Johnston, G. A. R., in *Handbook of Neurochemistry* (edit. by Lajtha, A.), 4, 115–134 (Plenum Press, New York 1970).
- Davison, A. N., and Kaczmarek, L. K., *Nature*, **234**, 107–108 (1971).
- Iversen, L. L., Mitchell, J. F., and Srinivasan, V., *J. Physiol., Lond.*, **212**, 519–534 (1971).
- Obata, K., and Takeda, K., *J. Neurochem.*, **16**, 1043–1047 (1969).
- Dreifuss, J. J., Kelly, J. S., and Krnjevic, K., *Expl Brain Res.*, **9**, 137–154 (1969).
- Srinivasan, V., Neal, M. J., and Mitchell, J. F., *J. Neurochem.*, **16**, 1235–1244 (1969).
- Katz, R. I., Chase, T. N., and Kopin, I. J., *J. Neurochem.*, **16**, 961–967 (1969).
- Hammerstad, J. P., Murray, J. E., and Cutler, R. W. P., *Brain Res.*, **35**, 357–367 (1971).
- Hopkin, J., and Neal, M. J., *Br. J. Pharmacol.*, **42**, 215–223 (1971).
- Bradford, H. F., *Brain Res.*, **19**, 239–247 (1970).
- Iversen, L. L., *The Uptake and Storage of Noradrenaline in Sympathetic Nerves*, 233–235 (Cambridge University Press, London, 1967).
- Hamberger, A., *Brain Res.*, **31**, 169–178 (1971).
- Levi, G., Blasberg, R., and Lajtha, A., *Archs Biochem. Biophys.*, **114**, 339–351 (1966).
- Baldessarini, R. J., and Kopin, I. J., *Science*, **152**, 1630–1631 (1966).
- McIlwain, H., and Rodnight, R., *Practical Neurochemistry*, 112–118 (Little, Brown, Boston, 1962).
- Orrego, F., and Lipmann, F., *J. Biol. Chem.*, **242**, 665–671 (1967).
- Keese, J. C., Wallgren, H., and McIlwain, H., *Biochem. J.*, **95**, 289–300 (1965).
- Schultz, S. G., and Curran, P. F., *Physiol. Rev.*, **50**, 637–718 (1970).
- Keese, J. C., and Wallgren, H., *Biochem. J.*, **95**, 301–310 (1965).

²⁰ Frankenhaeuser, B., and Hodgkin, A. L., *J. Physiol. Lond.*, **137**, 218–244 (1957).

²¹ Frankenhaeuser, B., *J. Physiol. Lond.*, **137**, 245–260 (1957).

Insulin unmasks latent sodium pump sites in frog muscle

INSULIN stimulates the movement across the cell membrane of a variety of substances such as glucose¹, amino acids², and alkali cations Na⁺ and K⁺ (refs 3 and 4). All these substances move through specific membrane sites but it is not known whether insulin stimulates transport by increasing the available number of sites or by increasing the efficiency with which the sites operate.

The alkali cation transport system, the Na-K ATPase, is well suited to explore this problem. The Na-K ATPase is selectively inhibited by ouabain^{5,6}. By the use of ³H-ouabain it is possible to determine the extent of the binding of the inhibitor and estimate the number of transport sites, while the alkali cation transport can be assayed by measuring the transport of labelled cations.

We have studied the effects of insulin on ³H-ouabain binding and ²²Na efflux of frog muscle. Paired sartorii dissected from the same frog (*Rana pipiens*) were used to provide control and test muscles. The techniques used to measure the efflux of labelled sodium have been described several times^{7,8}. An apparatus that transfers the muscles automatically from container to container

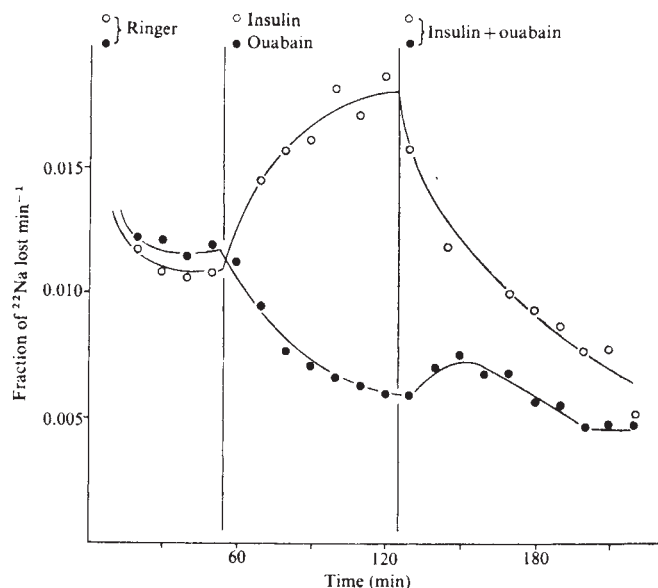


Fig. 1 The effects of ouabain on the stimulation of sodium efflux caused by insulin. After a control period the control muscle (●) received ouabain (1×10^{-6} M) and the test muscle (○) insulin (250 mU ml^{-1}). Then both muscles were transferred to solutions containing both ouabain and insulin.

was used⁹. ³H-ouabain (New England Nuclear) binding was measured in the following manner: after equilibrating the muscles in the labelled solution for 50 min, they were transferred through six containers, 10 min in each, containing ouabain-free Ringer solution at 0° C. Insulin-treated muscles were incubated in Ringer with insulin for 1 h before being exposed to a solution containing both insulin and ³H-ouabain. Excess fluid was removed and the muscles were weighed in a Mettler H10 balance. Then they were digested with NCS and counted in a toluene mixture on a Beckman LS150 liquid scintillation counter. Quenching was determined by the External Standard-Channels Ratio method. In all experiments the concentration of insulin used was 250 mU ml^{-1} .

Figure 1 shows the stimulation of sodium efflux caused by insulin and its inhibition by ouabain. The inhibitor reduced sodium efflux in the control muscle to a level equal to 0.50 times the resting value. This is in agreement with previous observations which show that only about half of the Na efflux in frog muscle is ouabain sensitive; the remaining efflux consists mostly of an exchange diffusion process^{7,10,11}. The subsequent addition of insulin resulted only in a small and transient increase in sodium efflux. In the test muscle insulin increased sodium efflux to 1.86 ± 0.10 times ($n = 5$) the control level. After the addition of ouabain, the efflux dropped to a level similar to that observed in the control muscle.

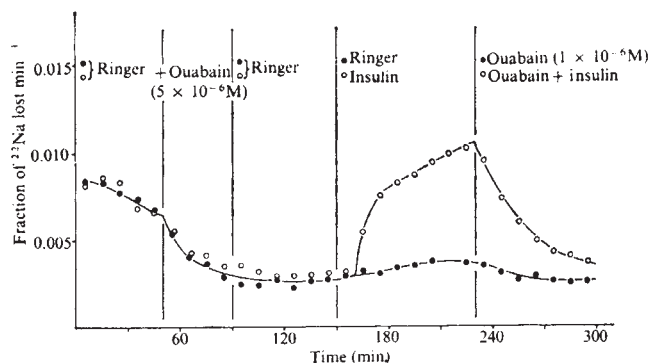


Fig. 2 First both muscles were immersed in 5×10^{-6} M ouabain solution, then washed in normal Ringer for 60 min. The test muscle (○) was then transferred for 80 min into a solution containing insulin and finally ouabain 1×10^{-6} M was added to the insulin containing solutions. The washout of the control muscle (●) was continued in control Ringer until 140 min were completed, then ouabain (1×10^{-6} M) was added.

The difference in ouabain binding between paired control muscles was always less than 5%. In the experiments on the effects of insulin on ³H-ouabain binding, we used two concentrations of ouabain, 2.5×10^{-7} M and 1×10^{-6} M, which inhibit 40% and 100% of the ouabain sensitive efflux, respectively.

Table 1 shows that insulin produced a large increase in ouabain binding. The ratios of ouabain binding by insulin treated over control muscles averaged 1.7. The average difference in binding between control and insulin treated muscles was significant for both concentrations of ouabain. The scatter in the binding figures arises because there are substantial differences in the amount of ouabain bound by muscles obtained from different frogs.

The stimulation of both sodium efflux and ouabain binding strongly suggests that insulin may increase the number of active sodium pumping sites in the muscle membrane. To test this possibility further, we performed some experiments based on the observation that the inhibition of the Na-K pump of frog muscle caused by ouabain is very slowly reversible^{12,13}. Thus one can block all the pumping sites in a resting muscle, then transfer it to an ouabain-free solution and determine whether insulin will still increase sodium pumping. Figure 2 shows one out of five experiments performed following this scheme. After a control period, the muscles were transferred to solutions containing ouabain (5×10^{-6} M). This concentration abolished the ouabain-sensitive component of sodium efflux. The muscles were then washed for 60 min in ouabain-free Ringer solution. This treatment does not remove ouabain from specific sites, since there is no recovery of the pump. The small increase in sodium efflux observed in Fig. 2 after washing the control muscle for 140 min in ouabain-free Ringer is the largest recovery we have observed in these and another series of experiments¹². In the five control muscles of the present series, the ratio of the efflux value measured after washing in ouabain-free Ringer for 140 min over the lowest value measured during the action of ouabain was 1.06 ± 0.05 . When insulin was added to the muscle a large increase of sodium

Table 1 Effects of insulin on ^3H -ouabain binding

Ouabain concentration	$2.5 \times 10^{-7} \text{ M}$	$1 \times 10^{-6} \text{ M}$	'Preincubated' $1 \times 10^{-6} \text{ M}$
Control	1661 $\pm$ 304	4037 $\pm$ 554	1153 $\pm$ 325
Insulin	2735 $\pm$ 499	6639 $\pm$ 825	2700 $\pm$ 336
Δ (I-C)	1074 $\pm$ 218	2602 $\pm$ 683	1546 $\pm$ 91
	$P < 0.01$	$P < 0.02$	$P < 0.001$
I/C	1.66 $\pm$ 0.08	1.71 $\pm$ 0.21	3.03 $\pm$ 0.66

All binding figures are expressed in d.p.m. per mg/wet weight. Values are averages $\pm$ s.e. of the mean ($n = 5$). The specific activity of ouabain was $11.7 \text{ Ci mmol}^{-1}$. Δ (I-C), The difference in binding between insulin-treated and control muscles; P values were calculated using Student's paired t test. I/C, The ratio of the binding of the insulin treated over the control muscles.

efflux was observed. The increase was equal to 0.76 ± 0.10 times ($n = 5$) the resting level. This stimulation of sodium efflux was inhibited when ouabain ($1 \times 10^{-6} \text{ M}$) was added to the washout solutions.

Another group of experiments of the same design as those illustrated in Fig. 2 was carried out to measure ouabain binding after saturating the sites available in resting muscle with unlabelled ouabain. They are summarised in Table 1 (preincubated). After immersing both the control muscles and test muscles in $5 \times 10^{-6} \text{ M}$ unlabelled ouabain (40 min) they were washed in inhibitor-free Ringer. The test muscles were then equilibrated with insulin and during the final period $1 \times 10^{-6} \text{ M}$ ^3H -ouabain was added to the solutions. The control muscle also received ^3H -ouabain in the final period.

Table 1 shows that preincubation with unlabelled ouabain reduced ^3H -ouabain binding in control muscles to about 30% of the value observed in muscles that were exposed directly to ^3H -ouabain. The binding remaining after the muscles had received enough ouabain to block all the Na active transport, probably represents binding to sites not linked to the cation transport process, that is unspecific binding. Indeed our own measurements of ^3H -ouabain binding by control muscles (to be published in detail elsewhere) show that in muscle cells, as in other cell types¹⁴, ^3H -ouabain binding has two components, one associated with inhibition of Na pumping sites and another which represents nonspecific binding. Table 1 also shows that the addition of insulin to muscles preincubated with unlabelled ouabain resulted again in a large and very highly significant ($P < 0.001$) increase in ^3H -ouabain binding. The last two columns of Table 1 show that the increase in ^3H -ouabain binding caused by insulin was smaller in muscles preincubated in unlabelled ouabain than in muscles that were not preincubated with the unlabelled inhibitor. In principle one would expect that the increase in labelling after insulin treatment should be the same in both experimental groups. We believe that the quantitative disagreement with this expectation is due in great part to the scatter in ouabain binding between muscles obtained from different frogs mentioned previously, since the difference in insulin-induced binding between both groups is not statistically significant ($P > 0.1$).

In any case, the parallel augmentation in ouabain-sensitive Na efflux and ouabain binding in muscles whose resting Na pump sites had been blocked by preincubation in unlabelled ouabain suggests that both phenomena are linked and provide further support for the notion that insulin acts by increasing the number of sodium pumps in the muscle membrane.

It could still be argued that insulin destabilises the ouabain-enzyme complex. The dissociation of the complex would result in the termination of the inhibitory effects of ouabain on the sodium efflux and would make available for binding those sites previously occupied by unlabelled inhibitor. This possibility is ruled out by two observations. First, that ^3H -ouabain binding measured without previous exposure to unlabelled ouabain is stimulated by insulin and second, that after equilibrating four pairs of muscles with ^3H -ouabain for 50 min, the test muscles were washed for 60 min in solutions containing insulin while the paired control muscles were washed in Ringer solution. Washing

with insulin did not decrease the amount of bound ^3H -ouabain. The ratio of ^3H -ouabain remaining in the insulin-washed muscle over the Ringer-washed muscle was 1.008 ± 0.04 ($n = 4$).

Assuming that one ouabain molecule blocks one pump site; that inhibition of the pump is directly proportional to the number of ouabain molecules bound per cell and a surface: weight ratio of $0.415 \text{ cm}^2 \text{ mg}^{-1}$ in frog muscle⁸, we arrived at a number of 1,500 pumping sites per square micrometre of membrane of control muscles for both concentrations of ouabain shown in Table 1. The estimates of membrane area consider only the cylindrical surface area of the muscle; since it is possible that pump sites are also present in the transverse tubular system, the number of pump sites per unit area could be overestimated by a constant factor. The calculation involves also the assumptions that 90% of the binding was specific when $2.5 \times 10^{-7} \text{ M}$ ouabain was used and 70% was specific for $1 \times 10^{-6} \text{ M}$ ouabain. These figures were estimated from our measurements of the kinetics of ouabain binding by control muscles and following criteria developed from observations in HeLa cells¹⁴, since binding of ouabain shares many common features in both types of cells.

The most interesting conclusion of these experiments is that insulin stimulates sodium pumping by increasing the number of pumping sites in frog muscle. This increase in number of pumping sites could result either from unmasking of latent pump sites or from synthesis of new enzyme. We found that the increase in ouabain binding caused by insulin was unaffected by the protein synthesis inhibitor cycloheximide ($100 \mu\text{g ml}^{-1}$). This observation suggests that the latter possibility is not likely.

We thank Dr J. Aceves for suggestions during the course of this work.

S. GRINSTEIN
D. ERLIJ

*Departamento de Biología Celular,
Centro de Investigación y de Estudios Avanzados del
Instituto Politécnico Nacional,
Apartado Postal 14-740,
Mexico 14, DF*

Received March 11; revised June 11, 1974.

- Levine, R., Goldstein, M., Klein, S., and Huddleston, B., *J. biol. Chem.*, **179**, 985 (1949).
- Manchester, K. L., and Young, F. G., *Biochem. J.*, **75**, 487 (1960).
- Zierler, K. L., *Am. J. Physiol.*, **198**, 1066 (1960).
- Moore, R. O., *J. Physiol., Lond.*, **232**, 23 (1973).
- Schatzmann, H. J., *Helv. physiol. pharmac. Acta*, **11**, 346 (1953).
- Glynn, I. M., *Pharmac. Rev.*, **16**, 381 (1971).
- Horowitz, P., Taylor, J. W., and Waggoner, D. M., *J. gen. Physiol.*, **55**, 401 (1970).
- Keynes, R. D., and Swan, R. C., *J. Physiol., Lond.*, **147**, 591 (1959).
- García, M., Leblanc, G., and Erlj, D., *Pflügers Arch. ges. Physiol.*, **311**, 278 (1969).
- Keynes, R. D., and Steinhardt, R. A., *J. Physiol., Lond.*, **198**, 581 (1968).
- Erlj, D., and Leblanc, G., *J. Physiol., Lond.*, **214**, 327 (1971).
- Erlj, D., and Elizalde, A., *Biochim. biophys. Acta*, **345**, 49 (1974).
- Abeles, A. L., *J. gen. Physiol.*, **54**, 268 (1969).
- Baker, P. F., and Willis, J. S., *J. Physiol., Lond.*, **224**, 441 (1972).

Surface and cell membrane activities of leukocyte chemotactic factors

PROTEINS with an affinity for air-water interfaces may also show an affinity for hydrophobic sites in cell membranes. In exploring the basis for recognition in leukocyte chemotaxis, I and my colleagues have previously reported that proteins which excite a locomotor response in leukocytes do so by virtue of their possession of externally positioned nonpolar groups¹⁻³. A protein may possess such groups either because it has become denatured, because nonpolar groups have been conjugated to it, or because, like α_s - or β -caseins, its intrinsic content of nonpolar residues is too high for them all to be packed internally⁴. These proteins would be expected to be surface active and could possibly penetrate the phospholipid bilayer of a leukocyte to activate the cell

for chemotaxis in a way that a more hydrophilic protein could not. This paper presents evidence that the surface activity and the chemotactic activity of proteins are indeed related, and that the chemotactic response of the leukocyte can be modified using membrane-active enzymes such as phospholipase A. These findings support the suggestion that chemotactic factors interact with the phospholipid bilayer of the cell membrane.

Human serum albumin (HSA) (Behringwerke, Marburg, Germany) was denatured either by treatment at pH 12 overnight at 20° C followed by return to pH 7, or by reduction-alkylation with 2-mercaptoethanol (0.1 M) and iodoacetamide (0.02 M). These preparations were dialysed against Gey's solution, pH 7.2 for use and the degree of denaturation achieved was measured by difference spectroscopy against native HSA. Butyryl HSA and guanidyl HSA were prepared as described previously². Milk proteins including fractionated samples of

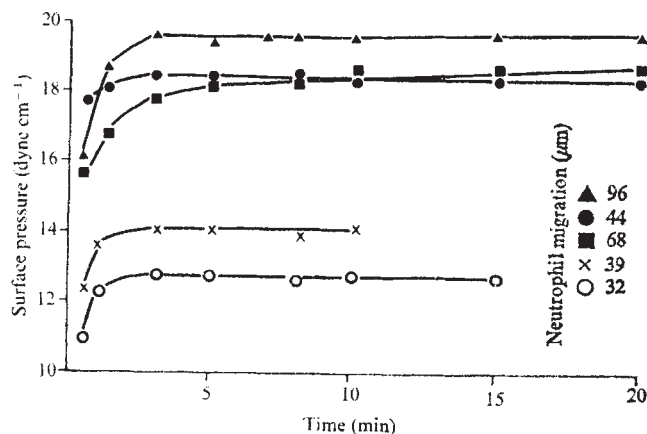


Fig. 1 Surface activity and chemotactic activity for neutrophils of native and modified human serum albumin (1 mg ml⁻¹). Both activities were measured on the same sample of each protein on the same day. The graph shows surface pressure measured against time after formation of the surface. The figures on the right represent the migration of neutrophils through a filter of 3 μm pore size towards each of the proteins, measured as the distance reached by the leading front of cells after 75 min at 37° C. ▲, Alkali-denatured HSA; ■, butyryl HSA; ●, reduced alkylated HSA; ×, guanidyl HSA; ○, HSA.

casein were obtained by courtesy of Dr R. L. J. Lyster, National Institute for Research in Dairying, Shinfield, Reading. Un-fractionated 'Hammarsten' casein was purchased from Merck A G, Darmstadt, Germany.

To obtain a direct comparison of the two activities in a physiological medium, surface pressure and chemotactic activity were both measured on the same sample of each protein at 1 mg ml⁻¹ in Gey's solution. The surface pressure at the air/Gey's solution interface was calculated from measurements of the force required to pull a glass plate out of the protein solution or Gey's solution at 20° C at timed intervals after pouring. Neutrophil leukocytes were obtained from normal human blood. The chemotactic assay was performed using modified Boyden chambers as described previously² and leukocyte migration within the micropore filter was measured by the leading front method of Zigmond and Hirsch⁵. The experimental conditions usually employed in Boyden-type assays do not distinguish directional from nondirectional locomotion of leukocytes. But using the modified assay systems described by Zigmond and Hirsch⁵, the proteins studied here have previously been shown⁶ to enhance both directional and nondirectional locomotion of leukocytes and may therefore legitimately be described as chemotactic factors.

Figures 1 and 2 show the surface pressures of various protein preparations and the migration of neutrophils towards the same preparations. Procedures which increased the surface activity of HSA (butyrylation, alkali denaturation) yielded products which attracted leukocytes more strongly than those showing smaller changes in surface activity, for example guanidyl-HSA (Fig. 1).

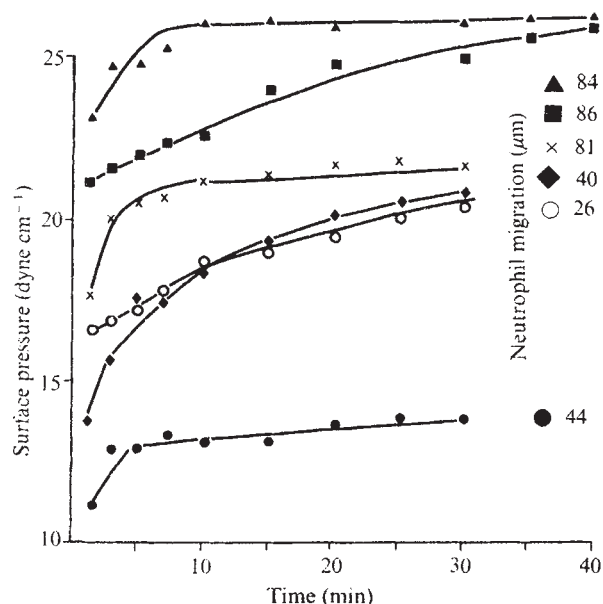


Fig. 2 Surface activity and chemotactic activity for neutrophils of fractionated milk proteins (each protein at 1 mg ml⁻¹). Both activities were measured on the same sample of each protein on the same day. Further details as in the legend to Fig. 1. ▲, β-casein; ■, whole casein; ×, α-casein; ◆, α-lactalbumin; ○, κ-casein; ●, β-lactoglobulin.

The data for milk proteins are shown in Fig. 2. Again the proteins (β-casein, α-casein and whole casein) which were most surface active also attracted neutrophils most strongly. Chemotactic substances may therefore be surface active. It is, however, important to note that not all surface active substances are chemotactic. Although the bee venom peptide, melittin, is highly surface active⁷, it does not attract leukocytes. Cells incubated with melittin (Sigma, 10⁻⁵ M to 10⁻⁶ M) and observed directly showed cytoplasmic movements for a few minutes and

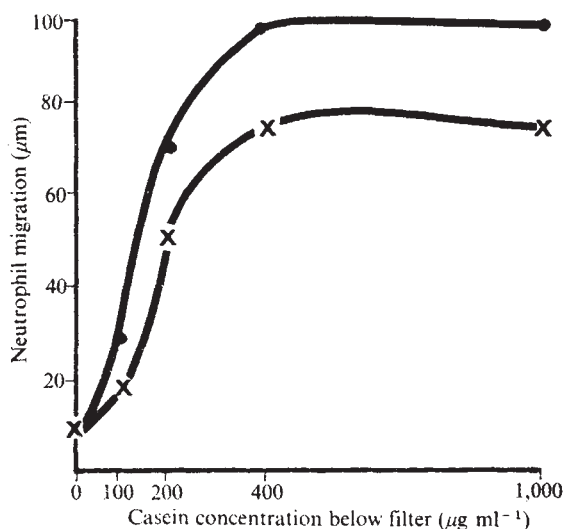


Fig. 3 Effect of phospholipase A on the migration of human neutrophils towards varying concentrations of whole casein. Healthy, freshly taken neutrophils (1 × 10⁶ ml⁻¹) were preincubated for 1 h at 37° C with whole casein (50 μg ml⁻¹) with or without bee venom phospholipase A (50 mU ml⁻¹ purified by Dr R. A. Shipolini, Department of Chemistry, University College London⁸). The cells and proteins were then added to the upper compartment of a Boyden chamber. The cells were allowed to migrate for 75 min towards casein at varying concentrations with (●) or without (×) phospholipase A (50 mU ml⁻¹) in the lower compartment. The phospholipase A alone had no effect on leukocyte migration. Provided that the neutrophils had not been spun in Ficoll-trisil, and that they were preincubated with the chemotactic factor as well as phospholipase, the result shown was reproducible.

then flattened out and sometimes lysed. At lower, nontoxic, concentrations there was no effect on locomotion. Chemotactic proteins probably require to interact with the cell membrane to initiate a locomotor response but without causing sufficient perturbation to damage the cell. Furthermore, small surface active molecules such as the nonionic detergent, Tween 80 (Atlas, Wilmington, Delaware) or the anionic detergent sodium dodecyl sulphate showed no chemotactic activity at non-cytotoxic concentrations, suggesting that chemotactic factors may require to be macromolecules.

The hypothesis that chemotactic proteins interact with the membrane bilayer is supported by preliminary studies of the influence of treatment with membrane active enzymes on the migration of leukocytes towards the substances discussed above. Treatment with phospholipase A enhanced the migration of neutrophils towards these substances (Fig. 3). This effect was observed using a highly purified enzyme from bee venom⁸ as well as with commercial preparations of phospholipase A derived from the venom of Russell viper (Koch-Light) and *Naja naja* (Koch-Light). To interact with the phospholipids of the cell membrane, phospholipase A has been shown to require the presence of direct lytic factor which is not present in pure enzyme preparations. But surface active agents⁹ and polypeptides with nonpolar groups¹⁰ may replace direct lytic factor and allow the phospholipase to reach its substrate. It is possible that chemotactic factors might act in the same way. Glycosidases (neuraminidase, β -glucosidase, β -galactosidase) had little effect on leukocyte migration. Trypsin at high doses (2 mg ml⁻¹) caused some reduction in migration.

These results suggest that the locomotor response of leukocytes to chemotactic factors is initiated by an interaction of the factor within the cell membrane bilayer itself, rather than with superficially placed structures, and that the relatively hydrophobic nature of these factors allows them to penetrate to interact at hydrophobic sites within the bilayer. This is supported by the enhancement of their chemotactic activity by phospholipase A which may facilitate entry of the chemotactic factor to the interior of the membrane. Interaction with a site deep in the membrane may be an essential preliminary step in initiating intracellular events such as microfilament contraction leading to locomotion. Since it has also been suggested that the ability of particles to be phagocytosed by neutrophils and macrophages may be determined by the relative hydrophobicities of the particle and of the cell which is to ingest it^{11,12}, it is possible that phagocytosis as well as locomotion is initiated by an interaction similar to that proposed here.

This work was supported by the Medical Research Council.

P. C. WILKINSON

Department of Bacteriology and Immunology,
University of Glasgow,
Glasgow G11 6NT, UK

Received March 18, 1974.

- ¹ Wilkinson, P. C., and McKay, I. C., *Int. Archs Allergy appl. Immun.*, **41**, 237-247 (1971).
- ² Wilkinson, P. C., and McKay, I. C., *Eur. J. Immun.*, **2**, 570-577 (1972).
- ³ Wilkinson, P. C., *Nature*, **244**, 512-513 (1973).
- ⁴ Waugh, D. F., Creamer, L. K., Slattery, C., and Dresdner, G. W., *Biochemistry*, **9**, 786-795 (1970).
- ⁵ Zimmond, S. H., and Hirsch, J. G., *J. exp. Med.*, **137**, 387-410 (1973).
- ⁶ Wilkinson, P. C., in *Primitive sensory and communication systems: the Taxes and Tropisms of Microorganisms and Cells* (edit. by Carlile, M. J.) (Academic Press, New York, in the press).
- ⁷ Sessa, G., Freer, J. H., Colacicco, G., and Weissmann, G., *J. biol. Chem.*, **244**, 3575-3582 (1969).
- ⁸ Shipolini, R. A., Callewaert, G. L., Cottrell, R. C., Doonan, S., Vernon, C. A., and Banks, B. E. C., *Eur. J. Biochem.*, **20**, 459-468 (1971).
- ⁹ Condrea, E., de Vries, A., and Mager, J., *Biochim. biophys. Acta*, **84**, 60-73 (1964).
- ¹⁰ Klibansky, C., London, Y., Frenkel, A., and de Vries, A., *Biochim. biophys. Acta*, **150**, 15-23 (1968).
- ¹¹ van Oss, C. J., and Gillman, C. F., *J. reticuloendothel. Soc.*, **12**, 283-292 (1972).

- ¹² Thrasher, S. G., Yoshida, T., van Oss, C. J., Cohen, S., and Rose, N. R., *J. Immun.*, **110**, 321-326 (1973).

B cell-derived immunoglobulin on activated mouse T lymphocytes

It is generally accepted that B lymphocytes express a high density of surface immunoglobulin (Ig). The presence of surface Ig on T lymphocytes, by contrast, is controversial. Thus, in mice, T cell surface Ig is considered to be undetectable by some workers¹⁻³; others, by contrast, report that T lymphocytes express as much surface Ig as B cells⁴.

Several groups have shown surface Ig on activated T cells (T-TDL) obtained from thoracic duct lymph of irradiated F₁ hybrid mice injected with parental strain thymus cells⁴⁻⁶. This communication presents evidence that a proportion of this Ig is of B cell origin.

T-TDL were collected from irradiated (800 r) (CBA×C57BL)F₁ hybrid mice injected 4-6 d previously with 2×10⁸ CBA or C57BL thymus cells⁷. Surface Ig on T-TDL was investigated by indirect immunofluorescence⁸. When T-TDL were collected 4-5 d after thymus cell injection, 49% were stained by purified rabbit anti-mouse Ig antibodies (Table 1). The staining was clearly discernible but was less intense than that shown by a small subpopulation (<0.3%) of the cells; the latter have been shown by other criteria to be B cells⁹. Most of the Ig detected on T-TDL collected at this stage was IgM. Staining with antisera against IgG₁, IgG_{2a} and IgG_{2b} was not above the control levels (3%) obtained with normal rabbit IgG. With T-TDL collected at 5-6 d, a higher proportion of the cells was Ig-positive (Table 1) and the intensity of staining was increased. IgM continued to be the predominant class, although most of the cells also expressed IgG.

The great majority of Ig-positive cells in T-TDL were undoubtedly T cells (95% are θ -positive). The surface Ig on T-TDL could have been synthesised by these cells; alternatively, it might have been derived from B cells, for example, those present in the thymocyte inocula from which the T-TDL were derived. To investigate this possibility, T-TDL were prepared from thymocytes depleted of B cells by passage through a Sephadex G-100 column conjugated with rabbit anti-mouse Ig antibody (modified from ref. 10). This procedure caused a 12-fold reduction in numbers of strongly Ig-positive (B) cells, that is, from 0.9% to 0.08%. No loss of B cells occurred when thymus cells were passed through control columns coated with normal rabbit IgG (NRIGG).

T-TDL derived from thymus cells filtered through a control column showed no changes in Ig staining relative to T-TDL derived from untreated thymocytes (see Tables 1 and 2). There was, however, a marked decrease in Ig-positive cells when the thymus cell precursors of T-TDL were passed through the anti-Ig immunoadsorbent column (Table 2). A higher proportion of T-TDL derived from B cell-depleted thymus were stained after incubation with NRIGG (Tables 2 and 3).

T-TDL depleted of surface Ig were still capable of specific antigen recognition. Thus, they were unimpaired in their ability to lyse ⁵¹Cr-labelled allogeneic tumour cells during a 6 h incubation period (Table 3). The presence of purified rabbit anti-mouse Ig antibody at a concentration of 1 mg ml⁻¹ did not significantly impair lysis (Table 3).

The above experiments clearly suggested that much of the IgM on T-TDL was derived from B cells in the thymus cell inocula used to prepare the cells. Direct evidence on the origin of the IgG on T-TDL was obtained with the use of an antiserum directed against allotype *a* of the Ig-1 locus expressed by IgG₁ and IgG_{2a} (G. F. Mitchell and L. Herzenberg, personal communication) (see legend to Fig. 1).

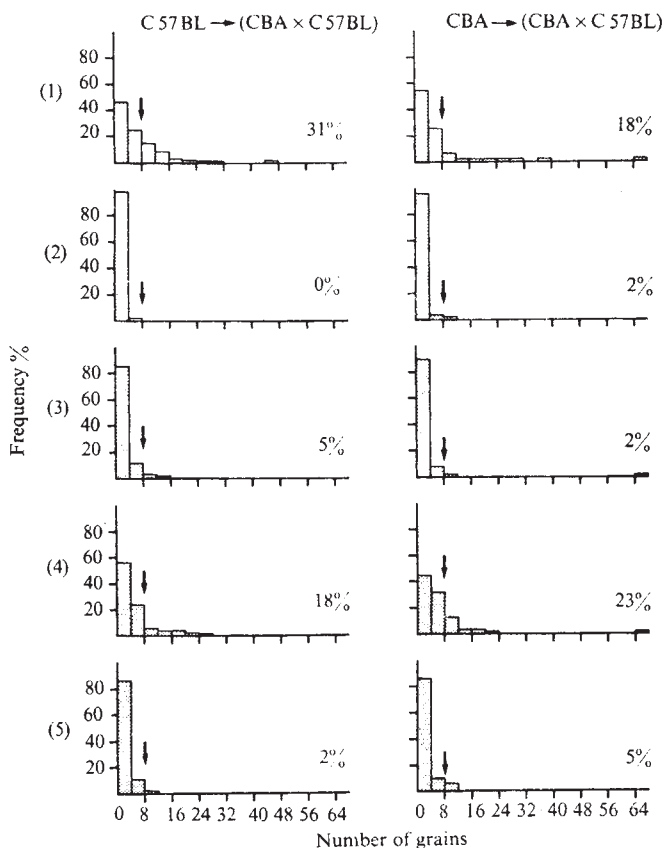


Fig. 1 T-TDL were generated by the transfer of 2×10^8 C57BL or CBA thymocytes in irradiated (800 r.) (C57BL $\times$ CBA) F_1 hybrids. T-TDL were collected 5–6 d after transfer. Aliquots of 10^7 CBA- or C57BL-derived T-TDL were incubated with one of the following: (1) 200 μ g of 125 I-labelled *b* anti-*a* allotype γ -globulin (125 I-*b* anti-*a* Ig); (2) uniodinated *b* anti-*a* Ig in a 5-fold excess for 30 min, and then 200 μ g of iodinated 125 I-*b* anti-*a* Ig; (3) a 5-fold excess of CBA γ -globulin (*a* Ig) in the presence of 200 μ g iodinated 125 I-*b* anti-*a* Ig; (4) a 5-fold excess of C57BL γ -globulin (*b* Ig) in the presence of 200 μ g iodinated 125 I-*b* anti-*a* Ig; (5) 200 μ g of 125 I-labelled normal C57BL γ -globulin (125 I-*b* Ig). All incubations were at 4°C for 30 min; cells were then freed of unbound γ -globulin by washing through discontinuous gradients of foetal bovine serum, and finally processed for autoradiography. After development, grain counts were performed on four coded slides per group; a minimum of 200 cells per group were counted. The percentage of cells bearing more than eight grains (indicated $\downarrow$) is shown in each group.

T-TDL bound this anti-*a* allotype serum irrespective of whether they were derived from CBA (allotype *a*) or C57BL (allotype *b*) thymus (Fig. 1). The binding of the labelled antibody was specific since it was blocked by pre-treatment with a five-fold excess on non-iodinated antibody and was reduced by the presence of CBA but not C57BL γ -globulin (Fig. 1); furthermore, it stained B cells from lymph nodes of CBA mice but not of C57BL mice (data not shown). These studies indicated that, in the case of T-TDL derived from C57BL thymus, part of the IgG detected on the cells was of recipient origin.

The demonstration of surface immunoglobulin on T-TDL by indirect immunofluorescence (Table 1) is in accord with the published data of other groups^{4–6}. It is probable that all T-TDL carry some surface Ig since staining using purified anti-Ig antibodies detected over twice as many Ig-positive blasts as an IgG fraction of the anti- κ serum (Table 1).

The data presented here suggest that the surface IgM, detected by immunofluorescence, was derived from donor B cells in the thymus cell suspensions from which the T-TDL were prepared.

The IgM on T cells might be adsorbed by the Fc binding site; this would be consistent with evidence that a high proportion of H2-activated T cells (ATC) from spleen form

Table 1 Immunoglobulin subclasses on normal T-TDL

T-TDL stained with	% Fluorescent staining ($\pm$ s.e.)	
	Day 4/5*	Day 5/6†
Anti-IgA	9 $\pm$ 3	7
Anti-IgM	34 $\pm$ 4	78
Anti-IgG ₁	4 $\pm$ 2	55
Anti IgG _{2a}	0.7 $\pm$ 0.7	8
Anti-IgG ₂	0.3 $\pm$ 0.3	12
Anti- κ light chains	23 $\pm$ 3	42
Anti-immunoglobulin antibodies	49 $\pm$ 8	55
Normal rabbit IgG	3 $\pm$ 1	10

* Number of days after thymus cell transfer; mean $\pm$ s.e. of three experiments.

† Results averaged from two experiments; duplicates within 13% of the mean.

2×10^8 CBA thymocytes were transferred to 800 r irradiated (CBA $\times$ C57BL) F_1 hybrid mice. T-TDL were collected 4/5 d after thymocyte transfer⁷ and were thoroughly washed with HEPES buffered Eagle's medium. Aliquots of 10^7 T-TDL were incubated for 30 min at 4°C with 100 μ g of the IgG fraction of various rabbit anti-mouse immunoglobulins⁸. Bound antibody was detected by a fluorescein-labelled goat anti-rabbit Fc serum. Cells were examined in suspension under a Leitz incident light ultraviolet microscope, and the percentage of positive cells determined for each coded preparation. A minimum of 200 cells were counted per preparation⁹. It is to be emphasised that the specificity of the antisera has already been established²⁰. They were titrated for specific immunofluorescence against lymph node and spleen cells. Furthermore, the staining seen on blasts with NRiG was never seen on small lymphocytes.

rosettes with sheep erythrocytes (SRBC) coated with anti-SRBC (an Fc dependent process)^{12,13}. If this were the sole mechanism, however, it would be paradoxical that T-TDL prepared from B-cell-depleted thymus cells showed decreased surface IgM, that is, one might have expected recipient Ig to replace the missing donor Ig. Significantly rosette formation by ATC from spleen was blocked by free IgG but not IgM. Thus if IgM is not cytophilic for T blasts, how are we to account for its presence on T-TDL? An intriguing possibility, analogous to that proposed by Crone *et al.*³, is that the donor IgM antibody might be bound not only to an Fc receptor but also by its combining site to soluble allo-antigen specifically captured by the endogenous T cell receptor. According to this view, donor B cells in the thymus inoculum would produce antibody against the recipient alloantigens; some of the antibody would bind to antigen already captured by the antigen-specific receptors on T-TDL; binding would be stabilised by the attachment of the antibody to the Fc receptor. Removal of donor B cells would thus remove the precursors of cells producing such antibody. This mechanism might explain the specific qualities of the T-TDL surface IgM isolated by Cone *et al.*¹⁴.

The IgG of host origin cannot be accounted for by this

Table 2 Staining of T-TDL after anti-Ig column pretreatment of progenitor thymocytes

Column	T-TDL staining with	% Fluorescent staining ($\pm$ s.e.)	
		Day 4/5*	Day 5/6†
Anti-Ig	Anti-Ig antibodies	12 $\pm$ 3	29
	IgG anti-IgM	15 $\pm$ 11	ND
	NRiG	7 $\pm$ 3	13
NRiG	Anti-Ig antibodies	49 $\pm$ 12	69
	IgG anti-IgM	62 $\pm$ 27	ND
	NRiG	8 $\pm$ 1	4

* Number of days after thymus cell transfer; mean $\pm$ s.e. of three experiments.

† Results averaged from two experiments; duplicates within 10% of the mean.

Aliquots of 10^7 CBA thymocytes were filtered through Sephadex G-100 columns to which rabbit anti-mouse Ig antibody or NRiG had been covalently attached¹⁰. 2×10^8 of these filtered cells were transferred to 800 r irradiated (CBA $\times$ C57BL) F_1 hybrids, and the T-TDL were collected 4/5 or 5/6 d after transfer. T-TDL were thoroughly washed with HEPES buffered Eagle's medium and stained for surface immunoglobulin as described in Table 1.

Table 3 Release of ^{51}Cr from DBA/2 mastocytoma cells by DBA/2-activated CBA T-TDL

Treatment of T-TDL cells	% Fluorescent staining of T-TDL with		Antibody in culture	% ^{51}Cr release	
	anti-Ig antibody	NRIGG		Exp. 1*	Exp. 2†
Filtered through NRIGG column	69	8	none anti-Ig NRIGG	94 87 93	59 55 61
Filtered through anti-Ig column	16	25	none anti-Ig NRIGG	94 85 88	62 60 64
Spontaneous release from tumour cells				19	9

* Killer: target ratio 20:1

† Killer: target ratio 10:1

CBA thymocytes were filtered through anti-Ig or NRIGG immunoadsorbent columns. 2×10^8 of these cells were transferred to 800 r irradiated (CBA $\times$ DBA/2) F_1 hybrids. T-TDL were collected 5/6 d after thymocyte transfer, and after washing, were mixed with ^{51}Cr -labelled P815Y mastocytoma cells at a ratio of 20:1 and 10:1¹⁴. The cell mixtures were incubated at 37°C for 6 h and the % ^{51}Cr release then counted. In addition, 1 mg ml⁻¹ of rabbit anti-mouse Ig antibody or NRIGG was added to identical mixtures throughout the incubation period.

mechanism since the F_1 (recipient) cells presumably cannot recognise the donor (parental) antigens. This IgG, but not donor-derived IgG, is therefore probably acquired by Fc receptors. It has not yet been possible, however, to obtain direct evidence that Fc receptors are expressed by T-TDL⁹.

How can the discrepancy between these findings with ATC-spleen and T-TDL be explained? According to the model considered above, alloantigen bound to endogenous T cell receptors could act as an immunoadsorbent to facilitate the capture of specific antibody; the Fc portion of this antibody might preferentially come to occupy the Fc receptors on the cell, thus saturating these receptors and preventing nonspecific uptake of extraneous Ig. It is possible that the splenic architecture may allow T blasts (ATC) to be formed in an environment relatively free of alloantibody, for example in the T-dependent areas. ATC collected from the spleen would therefore have free Fc receptors. Subsequent migration of these cells through alloantibody producing areas, for example B-dependent areas, before their eventual appearance in the lymph, would saturate the Fc receptors with alloantibody. It would follow, therefore, that Fc receptor blockade would be less evident on T-TDL derived from B cell-depleted thymus cells. Though this prediction has yet to be verified, it is consistent with the finding that, unlike normal T-TDL, a high proportion of T-TDL derived from B cell-depleted thymocytes bound NRIGG (Tables 2 and 3).

The mechanism proposed for specific antigen and antibody capture by activated T cells can be invoked to explain two phenomena observed by other workers.

(1) T rosette-forming cells (T-RFC) to SRBC from mice can, unlike most other T-cell reactions, be blocked by anti-Ig chain and anti-IgM sera. It is suggested that such immune rosettes are formed by specifically activated T cells binding a complex of antigen and specific IgM antibody, the Fc of the immunoglobulin stabilising the binding of antigen and the free combining sites of the antibody being available to bind the RBC used to form rosettes¹⁵.

(2) Bursectomised chickens are deficient in cells forming specific rosettes with SRBC. But preinjection of these birds with an anti-SRBC serum restores the numbers of RFC to the levels found in normal chickens¹⁶. It is possible that the antiserum used here contained antigen-antibody complexes in antibody excess. If so, then according to the model considered above, the complexes would bind only to specific T cells: the antigen attaching to the endogenous receptors of the cells and being held there by Fc binding of the

antibody. The free binding sites of the antibody would be available to form rosettes.

The possible role of this mechanism in initiating or aiding cellular cooperation *in vivo* has been recently reviewed by Playfair¹⁷.

Despite the evidence presented here and in other studies^{18,19} that much of the surface Ig on T cells is of exogenous origin, the proposed mechanism for stabilising antigen-binding by T cells requires that these cells express antigen-specific receptors. These may or may not be immunoglobulin. But it is clear that in all experiments attempting to demonstrate endogenous synthesis of immunoglobulin by T cells, one must consider the possible presence of B cell-derived immunoglobulin.

We thank Miss Nazra Thantrey and Miss Patricia Young for technical assistance, and Miss Penny Hamilton for preparing the manuscript. Drs G. F. Mitchell and L. Herzenberg kindly provided the anti-allotype serum. L. H. was supported by grants from the Medical Research Council of Great Britain. J. F. A. P. M. was supported by grants from the National Health and Medical Research Council of Australia, the Australian Research Grants Committee, the Damon Runyon Memorial Fund for Cancer Research and the Australian Kidney Fund.

Note added in proof: As a corollary to the above model, if activated T cells are generated in situations where alloantibody is not produced, as for example between mice differing only at the M locus, immunoglobulin should not be demonstrable on the surface of the activated cells. This has been shown experimentally to be the case (Hudson, L., Sprent, J. and von Boehmer, H., unpublished data).

L. HUDSON

Department of Immunology,
Middlesex Hospital Medical School,
London, UK

J. SPRENT

Basel Institute for Immunology,
CH-4058 Basel, Switzerland

J. F. A. P. MILLER

Walter and Eliza Hall Institute,
Melbourne, Australia

J. H. L. PLAYFAIR

Middlesex Hospital Medical School

Received February 4; revised April 10, 1974.

¹ Vitetta, E. S., Bianco, C., Nussenzweig, J. W., and Uhr, J. W., *J. exp. Med.*, **136**, 81 (1972).

² Raff, M. C., *Immunology*, **19**, 637 (1970).

³ Crone, M., Koch, C., and Simonsen, M., *Transplant Rev.*, **10**, 36 (1972).

⁴ Marchalonis, J. J., Cone, R. E., and Atwell, J. L., *J. exp. Med.*, **135**, 956 (1972).

⁵ Bankhurst, A. D., Warner, N. L., and Sprent, J., *J. exp. Med.*, **134**, 1005 (1971).

⁶ Pernis, B., Miller, J. F. A. P., Forni, L., and Sprent, J., *Cell. Immun.* (in the press).

⁷ Sorent, J., and Miller, J. F. A. P., *Cell. Immun.*, **3**, 385 (1972).

⁸ Hudson, L., and Roitt, I. M., *Eur. J. Immun.*, **3**(2), 63 (1973).

⁹ Basten, A., Miller, J. F. A. P., Sprent, J., and Pye, J., *J. exp. Med.*, **135**, 610 (1972).

¹⁰ Schlossman, S. F., and Hudson, L., *J. Immun.*, **110**, 313 (1973).

¹¹ Brunner, K. T., Mauel, J., Cerottini, J. C., and Chapuis, B., *Immunology*, **14**, 181 (1968).

¹² Yoshida, T. O., and Andersson, B., *Scand. J. Immun.*, **1**, 401 (1972).

¹³ Soteriades-Vlachos, C., Gyöngyösy, M., and Playfair, J. H. L., *Clin. exp. Immun.* (in the press).

¹⁴ Cone, R. E., Sprent, J., and Marchalonis, J. J., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2556 (1972).

¹⁵ Hudson, L., Greenberg, A. H., Roitt, I. M., and Bach, J. F., *Scand. J. Immun.*, **2**, 425 (1973).

¹⁶ Webb, S. R., and Cooper, M. D., *J. Immun.*, **111**, 275 (1973).

¹⁷ Playfair, J. H. L., *Clin. exp. Immun.* (in the press).

¹⁸ Hudson, L., Thantrey, N., Roitt, I. M., and Payne, L. N., *Immunology* (in the press).

¹⁹ Hunt, S., and Williams, A., *J. exp. Med.*, **139**, 479 (1974).

²⁰ Jones, G., Torrigiani, G., and Roitt, I. M., *J. Immun.*, **106**, 1425 (1971).

Removal of specific cooperative T cell factor by anti-H-2 but not by anti-Ig sera

ANTIBODY production to thymus-dependent antigens requires cooperation between thymus derived (T) and bone marrow derived (B) lymphocytes¹. B cells are the precursors of the antibody producing cells, but they apparently require the stimulatory influence of T cells in order to be triggered to proliferate and differentiate into plasma cells. The nature of the cooperative stimulus which passes from T cell to B cell is not yet established, though several possibilities have been discussed¹⁻³. There is evidence that T cells produce various mediators which may stimulate B cells, including nonspecific as well as antigen-specific T cell products.

One of us has recently described the production of an antigen-specific factor by specifically activated or 'educated' mouse T cells⁴. The factor is capable of replacing T cells in a co-operation dependent antibody response *in vivo*. We have attempted to identify this T cell product by removing it with various antisera immobilised as immunoadsorbents. The results described here show that the factor could not be removed by a sheep anti-mouse immunoglobulin (Ig) adsorbent, but was removed completely by congenically raised anti-H-2 antibodies.

The production and activity of the specific cooperative T cell factor have been described in detail in a previous report⁴. The antigen used was the multichain synthetic polypeptide poly(Tyr, Glu)-polyDLAla—polyLys, abbreviated as (T,G)-A—L (ref. 5), supplied by Dr Edna Mozes (Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot, Israel). Mice used were of the strains BALB/c, C57BL/10ScSn (B10) and strains congenic with B10, namely B10.D2, B10.Br and B10.A. All strains were obtained from the Laboratory Animal Centre, Carshalton, and were bred at the Department of Pathology, Tennis Court Road, Cambridge. In brief, T cells, primed or educated to (T,G)-A—L *in vivo*, were incubated together with antigen *in vitro* for 6–8 h. The cells were then removed by centrifugation and the supernatant, containing the T cell factor, transferred together with bone marrow cells and (T,G)-A—L into lethally irradiated, syngeneic recipients. After 14 d the direct plaque forming cell (PFC) response to (T,G)-A—L in the spleens of the recipients was measured and compared with controls receiving B cells and antigen but no factor, or B cells with T cells and antigen.

Table 1 Inability to remove T cell factor by anti-mouse Ig adsorbent

Cells and factors transferred	Antigen	Mean* PFC per spleen
B cells†	(T, G)-A—L‡	1.699
B cells + T cells§	(T, G)-A—L	3.805
B cells + T cell factor¶	(T, G)-A—L	3.820
B cells + T cell factor after adsorption by		
(1) normal sheep globulin	(T, G)-A—L	3.778
(2) sheep anti-mouse Fab'	(T, G)-A—L	3.792
Standard error		0.176

* log₁₀ geometric means.

† 10⁷ bone marrow cells.

‡ 10 µg in solution.

§ 10⁸ normal thymocytes.

¶ Produced by one spleen equivalent educated T cells.

Direct anti-(T, G)-A—L PFC responses in irradiated (750 r) BALB/c mice 14 d after receiving combinations of syngeneic B cells, T cells and T cell factor, adsorbed as indicated.

Antisera used for absorption of the factor were raised as follows. Sheep anti-mouse Fab' was prepared by immunisation with the Fab' fragment of BALB/c myeloma protein AdjPc5 (the myeloma was originally provided by Dr Michael Potter). First inoculation was given in Freund's complete adjuvant, followed by several inoculations in solution. The alloantisera used were anti-H-2^d, anti-H-2^k and anti-H-2^b, and were raised in strains congenic with those producing the factor under test. Anti-H-2^d was prepared by repeated weekly inoculation of DBA/2 spleen cells into B10.Br (H-2^k) mice; anti-H-2^k

was prepared by repeated weekly inoculation of CBA spleen cells into B10.D2 (H-2^d) mice; and anti-H-2^b by repeated weekly inoculation of either C3H.WB or B10 spleen cells into B10.A (H-2^a) mice. All the sera were cytotoxic, in the presence of complement, for lymphocytes of the appropriate H-2 type, with the exception of one of the anti-H-2^b sera (B10 cells into B10.Br). Immunoadsorbents were prepared from these sera, as well as from normal mouse serum, by precipitating the gamma globulin with 45% (NH₄)₂SO₄ and coupling to activated sepharose after removal of the (NH₄)₂SO₄⁶. T cell factor was tested immediately after preparation by passage over the immunoadsorbents in the form of short columns, and was then mixed with B cells and (T,G)-A—L and transferred into irradiated recipients as above.

Table 2 Removal of T cell factor by anti-H-2 adsorbent

Cells and factors transferred	Antigen	Mean* PFC per spleen
B cells†	(T, G)-A—L‡	2.176
B cells + T cells§	(T, G)-A—L	4.212
B cells + T cell factor¶	(T, G)-A—L	4.107
B cells + T cell factor after adsorption by		
(1) normal mouse globulin	(T, G)-A—L	4.130
(2) anti-H-2 ^d	(T, G)-A—L	2.220
(3) anti-H-2 ^k	(T, G)-A—L	4.198
Standard error		0.145

* log₁₀ geometric means.

† 10⁷ bone marrow cells.

‡ 10 µg in solution.

§ 10⁸ normal thymocytes.

¶ Produced by one spleen equivalent educated T cells.

Direct anti-(T, G)-A—L PFC responses in irradiated (850 r.) B10.D2 (H-2^d) mice 14 d after receiving combinations of syngeneic B cells, T cells and T cell factor. The factor, produced by B10.D2 T cells, was adsorbed as shown.

The first results show an attempt to remove the T cell factor by passage over a sheep anti-mouse Fab' column (Table 1). The factor, which was in this case prepared from BALB/c T cells educated to (T,G)-A—L, emerged from such a column with undiminished ability to cooperate with B cells in the response to (T,G)-A—L *in vivo*. The ability of the anti-Fab' column to bind mouse Ig was established using radiolabelled mouse Ig. The conclusion may be drawn from this experiment that the nature of the cooperative T cell factor is not that of conventional mouse Ig, at least in so far as it does not seem to carry mouse κ chains, the predominant light chain type of mouse Ig. But since the sheep serum was raised against a mouse myeloma Fab', various other possibilities still remain open, though they are rather unlikely, for example the T cell factor carries λ rather than κ chains, or is composed of heavy chains without light chains. With these reservations, and within the limitation that only one anti-mouse Ig serum has been used in this work so far, the result shown in Table 1 strongly suggests that T cell factor is not Ig. This finding also rules out any remaining suspicion that the T cell factor may be a B cell contaminant.

Attempts were then made to remove the T cell factor, with immunoadsorbents prepared from alloantisera. Congenic strains derived from the B10 genotype and differing only in the major histocompatibility locus, H-2, were used. Table 2 shows the results of an experiment in which T cell factor was prepared in the B10.D2 strain (H-2^d) and passed through immunoadsorbents of either anti-H-2^d or anti-H-2^k specificity, or normal mouse globulin. The factor was totally removed by the anti-H-2^d adsorbent, but not at all by anti-H-2^k or normal mouse globulin. Since the anti-H-2^d serum was prepared in a congenic strain (by injection of DBA/2 spleen cells into B10.Br mice), it is very unlikely to have reacted with any B10.D2 antigens other than those in the H-2 complex. The result thus implies that the T cell factor carries alloantigenic determinants of the T cells from which it is produced. This finding was confirmed in another strain, B10 (H-2^b) (Table 3). T cell factor was prepared from B10 T cells educated to (T,G)-A—L,

and passed through adsorbents containing anti-H-2^b or anti-H-2^d. Two anti-H-2^b adsorbents were employed, both prepared from alloantisera raised in the congenic B10.A (H-2^a) strain, one by inoculation of C3H.WB spleen cells and the other by inoculation of B10 spleen cells themselves. Both these adsorbents completely removed the T cell factor produced by B10 educated T cells. In contrast, the anti-H-2^d adsorbent which successfully removed the factor produced by B10.D2 T cells did not adsorb that produced by B10 T cells (Table 3). It may be noted that the latter result rules out the possibility that the anti-H-2 sera adsorb the factor because they contain antibodies cross-reacting with (T,G)-A—L itself, to which the factor may be bound⁷. An anti-(T,G)-A—L adsorbent itself also fails to remove the factor (personal observation).

Table 3 Removal of T cell factor by anti-H-2 adsorbent

Cells and factors transferred	Antigen	Mean* PFC per spleen
B cells†	(T, G)-A—L‡	2.133
B cells + T cell factor§	(T, G)-A—L	4.170
B cells + T cell factor after adsorption by		
(1) anti-H-2 ^b ¶	(T, G)-A—L	2.176
(2) anti-H-2 ^b	(T, G)-A—L	2.112
(3) anti-H-2 ^d	(T, G)-A—L	4.225
Standard error		0.134

* log₁₀ geometric means.

† 10⁷ bone marrow cells.

‡ 10 µg in solution.

§ produced by one spleen equivalent educated T cells.

¶ C3H.WB spleen cells inoculated into B10.A mice.

|| B10 spleen cells inoculated into B10.A mice.

Direct anti-(T, G)-A—L PFC responses in irradiated B10 (H-2^b) mice 14 d after receiving combinations of B cells and T cell factor. The factor, produced by B10 T cells, was adsorbed as shown.

These results strongly suggest that the cooperative T cell factor is physically associated with alloantigens of the strain in which it is produced. The interpretation of these findings clearly depends on the specificities of the antibodies present in the alloantisera used. The alloantigens coded for by the H-2 region and expressed on the T lymphocyte surface include the products of the H-2K and H-2D regions, and in addition probably the products of the H-2-linked immune response (Ir) genes⁸. The latter control the levels of immune responses to specific antigens and in the mouse have been mapped in a region within the H-2 locus, between the H-2K and H-2D 'ends' and closer to H-2K⁸. There is evidence that Ir genes are functionally expressed in both T^{8,9} and B cells (refs 10–12 and G. M. Shearer, pages 38–39 in ref. 8). The finding that antisera to the H-2 complex remove the T cell factor may imply either that the factor is identical with the product of the H-2K or H-2D genes; or that it is an Ir gene product; or possibly that it is some as yet unidentified molecule which becomes physically attached to one of the H-2 products. Perhaps the most attractive speculation is that the factor consists of both the Ir and H-2K or H-2D gene products physically combined. The products of H-2K or H-2D might provide the 'constant' region of a molecule analogous to immunoglobulin, with Ir gene products providing the variation required for specific antigen binding. The use of recombinant strains, providing different combinations of H-2K, Ir and H-2D, should resolve this problem.

What is the relationship between T cell factor and T cell receptor? The most important feature of the T cell factor is its specificity for the inducing antigen⁴. By analogy with B cells, it is highly unlikely that T cells would produce a specific molecule that was different, in terms of antigen recognition, from the T cell receptor. It seems most probable, therefore, that the T cell factor is the soluble expression of the T cell receptor; that is, that the T cell factor and the T cell receptor are closely related molecules. The conclusion that the T cell receptor is intimately associated with H-2 region products finds considerable support in the work of others. Attempts to demonstrate Ig on the T cell surface have been largely un-

successful (refs 13, 14; E. Unanue, pages 207–220 in ref. 8; and J. W. Uhr, pages 228–238 in ref. 8), despite some positive reports^{15–18}. Anti-Ig serum fails to inhibit T cell functions such as mixed lymphocyte reaction (MLR) (I. Roitt, quoted in ref. 1, page 118) and graft-versus-host (GvH) reaction¹⁹, though antigen binding by T cells, helper activity and 'hot' antigen T cell 'suicide' have all been successfully inhibited by such sera^{20–22}, and a 7S IgM derived from activated T cells is able to cooperate with B cells *in vitro*³. The likelihood that T cells adsorb antibody on to their surface²³ makes interpretation of the positive results difficult against the background of widespread failure to show conclusively that Ig is the primary T cell receptor. The failure, reported here, to remove the T cell factor on an anti-Ig adsorbent also strongly suggests that the factor, and by inference the T cell receptor, are non-Ig in nature. On the other hand, positive association between T cell receptor and histocompatibility (H) antigens includes the blocking of MLR and PPD proliferative responses of human lymphocytes by anti-HL-A sera *in vitro*^{24,25}, the inhibition of the GvH response by alloantibodies¹⁹, and the abolition of the response of guinea pig T cells to specific antigens *in vitro* by antisera against H antigens²⁶. This last observation is clearly analogous to the absorption of T cell factor by alloantibodies, and it is interesting to note that in the guinea pig the active antibodies are apparently directed at H rather than Ir gene products²⁷. The authors, in this latter case, also conclude that H and Ir gene products must be linked physically.

A summary of our conclusions from the observations described here is as follows. H-2 region genes expressed by T cells are very probably responsible for antigen recognition by T cells. After recognising antigen, T cells release a soluble, antigen-specific factor which, in conjunction with antigen, is apparently able to transmit a stimulatory signal to B cells. The factor consists either wholly or partly of H-2 region gene products. This further suggests that the latter are important in the interaction with the B cell and (or) perhaps the macrophage.

The technical assistance of Rosalie Campbell, Heather Williams and Yvonne Lawson is gratefully acknowledged. This work was supported by grants from the Medical Research Council.

M. J. TAUSSIG
A. J. MUNRO

Immunology Division,
Department of Pathology,
Tennis Court Road,
Cambridge, UK

Received April 26; revised June 9, 1974.

¹ Greaves, M. F., Owen, J. J. T., and Raff, M. C., *T and B lymphocytes: Origins, Properties and Roles in Immune Responses* (Excerpta Medica, Amsterdam, 1973).

² Katz, D. H., *Transplant Rev.*, **12**, 141–179 (1972).

³ Feldmann, M., and Nossal, G. J. V., *Transplant Rev.*, **13**, 3–34 (1972).

⁴ Taussig, M. J., *Nature*, **248**, 234–236 (1974).

⁵ Sela, M., and Fuchs, S., in *Handbook of Experimental Immunology* (edit. by Weir, D. H.), **1**, chapter 1 (Blackwell Scientific, Oxford, 1973).

⁶ Fuchs, S., and Sela, M., in *Handbook of Experimental Immunology* (edit. by Weir, D. H.), **1**, chapter 1 (Blackwell Scientific, Oxford, 1973).

⁷ Ebringer, A., and Davies, D. A. L., *Nature new Biol.*, **241**, 144–147 (1973).

⁸ *Genetic Control of Immune Responsiveness* (edit. by McDevitt, H. O., and Landy, M.) (Academic Press, New York and London, 1972).

⁹ Benacerraf, B., and McDevitt, H. O., *Science*, **175**, 273–279 (1972).

¹⁰ Mozes, E., and Shearer, G. M., *Curr. Top. Microbiol. Immun.*, **59**, 167 (1972).

¹¹ Taussig, M. J., Mozes, E., and Isac, R., *J. exp. Med.* (in the press).

¹² Katz, D. H., Hamaoka, T., Dorf, M. E., Maurer, P. H., and Benacerraf, B., *J. exp. Med.*, **138**, 734–739 (1973).

¹³ Vitteta, E. S., Bianco, C., Nussenzweig, V., and Uhr, J. W., *J. exp. Med.*, **136**, 81–93 (1972).

¹⁴ Grey, H. M., Kubo, R. T., and Cerottini, J. C., *J. exp. Med.*, **136**, 1323–1328 (1972).

- ¹⁵ Hämmerling, U., and Rajewsky, K., *Eur. J. Immun.*, **1**, 447-452 (1971).
- ¹⁶ Bankhurst, A. D., Warner, N. L., and Sprent, J., *J. exp. Med.*, **134**, 1005-1015 (1971).
- ¹⁷ Nossal, G. J. V., Warner, N. L., Lewis, H., and Sprent, J., *J. exp. Med.*, **135**, 405-428 (1972).
- ¹⁸ Marchalonis, J. J., Cone, R. E., and Atwell, J. L., *J. exp. Med.*, **135**, 956-971 (1972).
- ¹⁹ Crone, M., Koch, C., and Simonsen, M., *Transplant. Rev.*, **10**, 36-56 (1972).
- ²⁰ Roelants, G. E., Forni, L., and Pernis, B., *J. exp. Med.*, **137**, 1060-1076 (1973).
- ²¹ Cheers, C., Breitner, J. C. S., Little, M., and Miller, J. F. A. P., *Nature new Biol.*, **232**, 248-250 (1971).
- ²² Basten, A., Miller, J. F. A. P., Warner, N. L., and Pye, J., *Nature new Biol.*, **231**, 104-106 (1971).
- ²³ Hunt, S. V., and Williams, A. F., *J. exp. Med.*, **139**, 479-496 (1974).
- ²⁴ Ceppellini, R., *Progr. Immun.*, **1**, 973 (1971).
- ²⁵ Buckley, R. G., Schiff, R. I., and Amos, D. B., *J. Immun.*, **108**, 34-44 (1972).
- ²⁶ Shevach, E. M., Paul, W. E., and Green, I., *J. exp. Med.*, **136**, 1207-1221 (1972).
- ²⁷ Shevach, E. M., Green, I., and Paul, W. E., *J. exp. Med.*, **139**, 679-695 (1974).

Isolation of a purified preparation of metabolically active retinal blood vessels

THE retinal vascular system has long been of interest to both clinicians and basic scientists because of its involvement in a number of systemic diseases, particularly hypertension and diabetes mellitus, and its accessibility to *in vivo* study through direct observation due to the transparency of the eye. This accessibility has made it an ideal system for the study of a portion of the microcirculation in various physiological and pathological states as well as under the influence of pharmacological agents. Such studies have advanced considerably the clinical and experimental investigation of retinal vascular disease which is one of the most common causes of visual disturbance and blindness in man. But *in vitro* study of retinal blood vessels has been restricted largely to the examination of retinal digest preparations obtained with the use of proteolytic enzymes¹ or by autolysis of the retina in water², and of isolated vessels obtained by microdissection techniques³. Metabolic studies on such preparations of isolated vessels have been extremely limited³ because of the non-physiological conditions used or their tedious and time consuming nature. Therefore, we have developed a simple procedure for the isolation of a purified preparation of bovine retinal vessels which are metabolically active.

Retinal vessels were obtained from freshly enucleated bovine eyes obtained at a local slaughter house. Immediately after removal from the animal, the eyes were bisected and the retinae removed and placed in ice-cold buffer consisting of Earle's balanced salt solution (116 mM NaCl, 16 mM KCl, 1 mM NaH₂PO₄·H₂O, 0.8 mM MgSO₄·7H₂O, 5.6 mM glucose, 1.8 mM CaCl₂) buffered with 28 mM HEPES (N-2-hydroxyethylpiperazine-N-2-ethane sulphonic acid (pH 7.4) and containing 0.01% bovine serum albumin, and 50 mg of penicillin G and streptomycin sulphate l⁻¹. The isolated retinae in buffer were carried back to the laboratory on ice where they were minced well with scissors and homogenised with 10 up and down strokes of a hand-held tapered Teflon pestle in a smooth glass tube. One part of tissue was homogenised in four parts of buffer (v/v). The homogenate was then poured over an 86 µm nylon sieve (Kressilk Products Inc., Monterey Park, California) and the sieve washed extensively with buffer. The material remaining on the sieve was washed off into a homogenisation tube and rehomogenised with five up and down strokes of the pestle. The resulting homogenate was again sieved through the

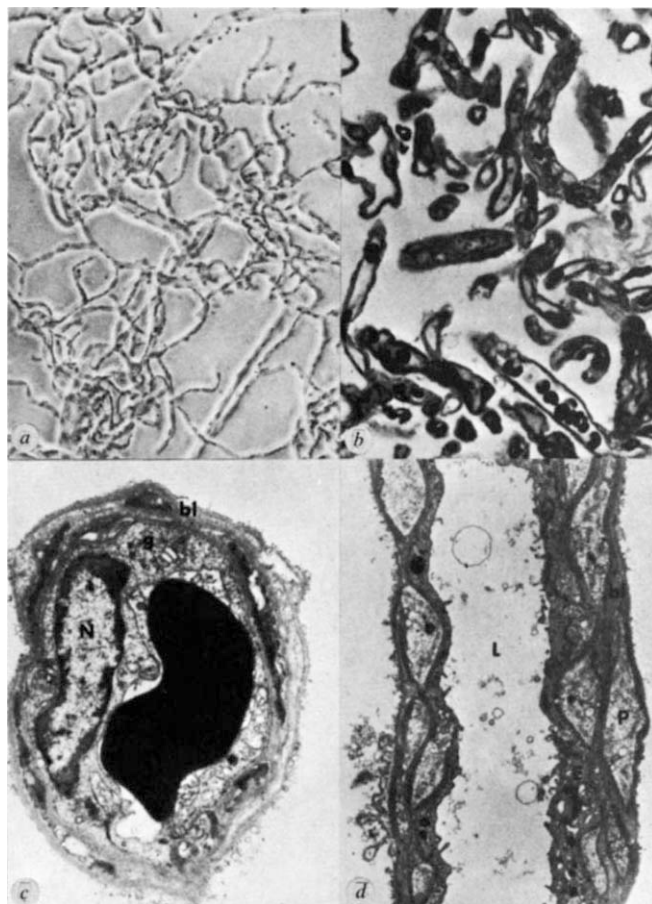


Fig. 1 *a*, Phase contrast photomicrograph of isolated bovine retinal vessel aggregate. Red blood cells are visible within vessel lumina. $\times 158$. *b*, Photomicrograph of epoxy-embedded isolated bovine retinal vessel aggregate. Vessels are cut in various planes of section. Non-vascular surrounding tissues are virtually absent. Toluidine blue, $\times 470$. *c*, Electron micrograph of cross section through isolated retinal vessel. A red blood cell occupies nearly the entire lumen. Endothelium is lined by basement lamina (bl) which surrounds cytoplasmic processes of adjacent pericytes. The endothelial cell exhibits a prominent nucleus (N) and Golgi apparatus (g). $\times 5447$. *d*, Electron micrograph of longitudinal section through isolated retinal vessel. The lumen (L) of the vessel is devoid of cells. A thin endothelium (E) lines this vessel and is surrounded by basement lamina (bl) which varies greatly in thickness and also surrounds intramural pericytes (P) $\times 3062$.

nylon sieve and the sieve washed thoroughly with buffer. The material now on the sieve consisted of a purified preparation of retinal blood vessels which were almost devoid of non-vascular tissue contamination with the exception of trapped blood elements, as determined by phase contrast and electron microscopy as well as by light microscopic observations of fixed tissue sections. The yield of vessels from the retinae of 18 cow eyes was 281 mg of wet weight tissue. If preparations enriched in large or small diameter vessels were desired, these could be obtained by first sieving the rehomogenised material through a 210 µm nylon sieve followed by sieving of the filtrate on an 86 µm sieve. The material found on the 210 µm sieve contained vessels of large and medium diameter with attached and adhering vessels of smaller diameter whereas that on the 86 µm sieve consisted almost entirely of vessels of smaller diameter. These preparations were washed off the sieves into centrifuge tubes and sedimented at 1,085g for 2 min to obtain a pellet of vessels which were resuspended in a suitable volume of buffer for metabolic experiments.

The metabolic activity of the vessel preparations was

determined by incubating 0.5 ml aliquots of a suspension of vessels with shaking at 37° C in an atmosphere of oxygen in the presence of appropriate ^{14}C -labelled substrate in a $^{14}\text{CO}_2$ collection apparatus described elsewhere⁴. Radioactivity determinations were performed in a Beckman model LS-250 scintillation counter using a scintillation fluid containing one third Triton X-100 (ref. 5). Protein was determined by the method of Lowry⁶. ^{14}C -1-oleic acid (specific activity 9.1 mCi mmol⁻¹) and ^{14}C -2, 3-succinic acid (specific activity 18.3 mCi mmol⁻¹) were products of New England Nuclear Corporation (Boston, Mass.). ^{14}C -6-D-glucose (specific activity 10 mCi mmol⁻¹) was obtained from Calbiochem (Los Angeles, California).

Phase contrast microscopy was carried out on freshly isolated vessel preparations using a Zeiss RA or Ultraphot II microscope. For other microscopic examination isolated blood vessels were fixed for 1 h in cold Karnovsky's⁷ fixative, buffered at pH 7.3. Postfixation was carried out in 2% OsO_4 buffered with 0.144 M sodium cacodylate. Tissues were dehydrated in a graded series of ethanols and propylene oxide followed by embedment in Epon-Araldite⁸. Thick (1 μm) and thin sections were cut with glass or diamond knives on a Porter-Blum-MT-2 Ultramicrotome. Thick sections were stained with toluidine blue for purposes of orientation and determination of vessel position. Thin sections were stained with uranyl acetate (5% in absolute ethanol) and lead citrate⁹. Light microscopic observations were made on a Zeiss Ultraphot II. Thin sections were

observed with a Philips EM-300 electron microscope at original magnifications of 1,200–13,000.

The technique which we describe for the isolation of retinal blood vessels results in a loose pellet of tissue which to the naked eye appears as a twisted aggregation of very fine thread-like tubules. By phase contrast microscopy (Fig. 1a) the pellet can be shown to be composed of an extraordinarily clean preparation of isolated blood vessels. Although the vessels exhibit a wide range of diameters (7–50 μm), most of them are approximately 10 μm in diameter. A few red blood cells are seen free in the dispersion medium, but the remaining tissues are composed of clearly recognisable vessels with enclosed blood elements. The remarkable homogeneity of these tissues is more clearly demonstrated by conventional light microscopy (Fig. 1b). Sections 1 μm thick of fixed tissue reveal an aggregate of vessels cut in longitudinal, cross and oblique section. Measurements of these vessels compare favourably with those seen in phase microscopy.

Electron microscopic observations of isolated retinal vessels show an extremely clean preparation. Nonvascular tissues consist largely of blood cells, some of which are found free in the embedding medium. Cross sections of capillaries (Fig. 1c) exhibit red blood cells within their lumina. The vessel wall is composed of endothelial cells and a basement lamina which may split to enclose cytoplasmic extensions of smooth muscle cells or intramural pericytes. Endothelial cell nuclei are surrounded by a smooth contoured nuclear envelope interrupted by nuclear pores. Many acanthosomes and micropinocytotic vessels are seen in the peripheral cytoplasm, and a prominent Golgi zone is frequently noted in the perinuclear area. Endothelial flaps adjacent to cellular junctions are frequent modifications of the luminal plasmalemma. In longitudinal section (Fig. 1d) the morphological integrity of the endothelium is preserved. Numerous mitochondria lie within the endothelial cell cytoplasm.

The maintenance of the subcellular compartmentation of the vascular cells of preparations isolated using this procedure was supported by their ability to metabolise ^{14}C -labelled oleic and succinic acids and ^{14}C -glucose to $^{14}\text{CO}_2$ (Fig. 2), metabolic transformations which cannot be carried out by red blood cells. This was confirmed by control experiments run under identical conditions with amounts of blood elements equivalent to those found entrapped in the incubations with vessels. In the experiment with vessels after an initial lag period, the oxidation of the above substrates was maintained at linear rates and continued at these rates for several hours demonstrating the integrity of the Embden-Myerhof pathway, the pathway of fatty acid oxidation and the Krebs cycle in this preparation. Thus, morphologically and metabolically, this is an intact purified preparation of isolated small blood vessels obtainable in good yield and suitable for analytical, biochemical and pharmacological studies. The isolation procedure avoids the difficulties involved in previously described procedures for the isolation of retinal vessels involving trypsinisation, autolysis of the retina in water and microdissection. Most retinal vessel preparations isolated using these techniques have been used for morphological^{1,2} and analytical^{10,11} studies and almost nothing is known concerning their metabolism. Metabolic studies have been limited to histochemical investigations¹² and experiments using the Cartesian diver³. Investigations of the metabolism of small blood vessels from nonretinal tissues have also been limited to studies of preparations obtained by dissection. Such preparations can be derived readily in adequate amounts only from non-mammalian sources such as the swim bladder of the eel¹³. The availability of a procedure which enables the quick and simple isolation of a purified preparation of small blood vessels in good yield from mammalian retinas should permit further investigations of the properties of this important type

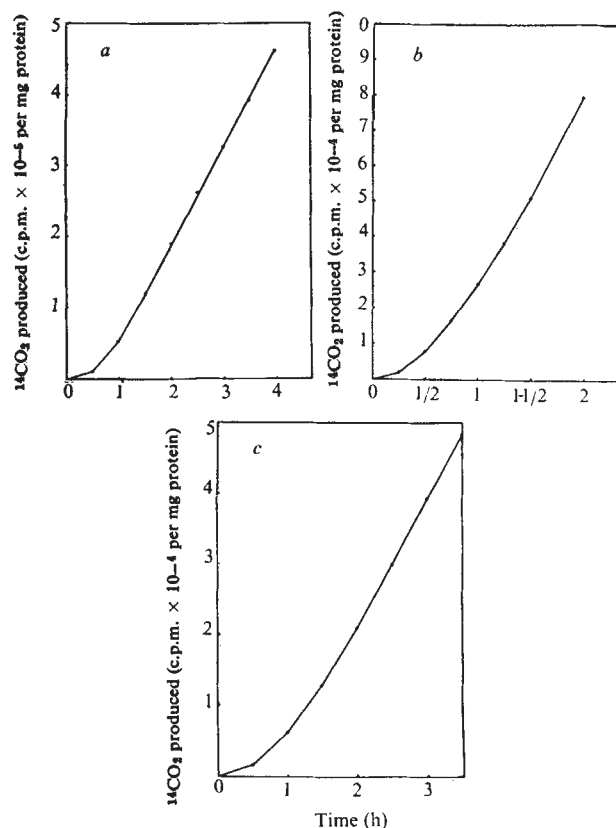


Fig. 2 Oxidative metabolism of several substrates by isolated retinal vessels: a, ^{14}C -6-D-glucose (0.25 μCi); b, ^{14}C -2,3-succinic acid (0.2 μCi); c, ^{14}C -1-oleic acid (0.2 μCi). Each incubation flask contained 83.5–236 μg of retinal vessel protein plus the designated amount of ^{14}C -labelled substrate in 0.51 ml of incubation buffer (minus glucose). Control experiments not shown in Fig. 2 include incubations with buffer alone and with bovine blood dilutions equivalent to blood elements entrapped in the corresponding vessel preparations (300–3,000 red blood cells). These controls yielded no significant $^{14}\text{CO}_2$ production over background from identical amounts of the above radioactive substrates.

of vascular tissue. This procedure has also been modified for the isolation of blood vessels from bovine cerebral cortex¹⁴ and may be adapted to the isolation of small blood vessels from other tissues.

We thank Dr Franz Matschinsky for helpful suggestions, Mary Lou Rankin and Rosalyn Upson for technical assistance, and R. C. Jones of Jones Meat Packers who generously provided the bovine eyes used in this study and Harlan Woodruff, Arizona State Meat Inspector.

ELIAS MEEZAN
KLAUS BRENDL

Department of Pharmacology

EDWARD C. CARLSON

Department of Anatomy,
University of Arizona Medical School,
Tucson, Arizona 85724

Received April 8, 1974.

- ¹ Kuwabara, T., and Cogan, D. G., *Arch. Ophthalmol.*, **64**, 904-911 (1960).
- ² Mutlu, F., and Leopold, I., *Arch. Ophthalmol.*, **71**, 93-101 (1964).
- ³ Howard, R. O., and Sears, M. L., *Invest. Ophthalmol.*, **3**, 432-440 (1964).
- ⁴ Brendel, K., and Meezan, E., *Analyt. Biochem.* (in the press).
- ⁵ Patterson, M. S., and Greene, R. C., *Analyt. Chem.*, **37**, 854-857 (1965).
- ⁶ Lowry, O. H., Rosebrough, N. S., Farr, A. L., and Randall, R. J., *J. Biol. Chem.*, **193**, 265-275 (1951).
- ⁷ Karnovsky, M. S., *J. Cell Biol.*, **27**, 137a-138a (1965).
- ⁸ Anderson, W. A., and Ellis, R. A., *J. Protozool.*, **12**, 483-499 (1965).
- ⁹ Venable, J. H., and Coggeshall, R., *J. Cell Biol.*, **25**, 407-408 (1965).
- ¹⁰ Futterman, S., and Kupfer, C., *Invest. Ophthalmol.*, **7**, 105-108 (1968).
- ¹¹ Heath, H., Paterson, R. A., and Hart, J. C., *Diabetologia*, **3**, 515-518 (1967).
- ¹² Levene, R. Z., Horton, G., and Einaugler, R., *Arch. Ophthalmol.*, **72**, 99-103 (1964).
- ¹³ Rasio, E. A., *Can. J. Biochem.*, **51**, 701-708 (1973).
- ¹⁴ Brendel, K., et al., *Science* (in the press).

Stimulation of ribosome synthesis during retarded ageing of human fibroblasts by hydrocortisone

CORTISONE¹ and hydrocortisone² can prolong the lifespan of human embryonic fibroblasts cultivated *in vitro* and this prolongation is accompanied by a significant increase in the saturation density. The same effect has been observed in cultures of primary human amnion cells³. One of the constant features during senescence *in vitro* of cells from different species is the progressive decrease of the cell density at which growth practically stops, and it has been suggested⁴ that the two phenomena, ageing *in vitro* and decrease of the saturation density, are related.

It is now well established that ribosome synthesis declines when division slows down during cell crowding⁵⁻¹⁰. It has also been reported that RNA synthesis declines during cell senescence¹¹. Since it has been shown that hydrocortisone stimulates RNA synthesis¹²⁻¹⁴ the increased saturation density and lifespan of cells carried in the presence of hydrocortisone could be due to a sustaining effect of the hormone on ribosome synthesis. We show here that, in fact, human fibroblasts carried in hydrocortisone-supplemented medium incorporate more radioactive precursors into ribosomes and polyribosomes than the control cultures. This effect may be correlated with the retarded ageing induced by the hormone *in vitro*.

A human embryonic fibroblastic line was cultured with and without hydrocortisone. The controls died at the 44th

passage and the hydrocortisone treated cells at the 62nd passage. At the 49th passage, cells carried in the hormone supplemented medium were subcultivated in medium without the hormone and died two passages later.

At the 36th passage part of the cultures from each group were pooled, subcultivated and counted each day until the growth curves reached a plateau. In addition, each day after subcultivation, cells from identical cultures were labelled and collected, and the cytoplasm extracts layered on 7-52% sucrose gradients (Fig. 1). According to previous data¹⁵ fractions 10-15 correspond to polyribosomes and fractions 16-25 to single ribosomes. Significantly more ribosomes and polyribosomes were synthesised on day 1 and day 2 in hydrocortisone-treated cells. During the third day, when the cultures exposed to hydrocortisone had already reached a plateau though the controls were still dividing, the radioactivity incorporated in the polyribosomal fractions was more or less identical in both groups (Fig. 1). On day 4 the cultures in both groups had reached a plateau and as expected, very few polyribosomes were synthesised and the radioactivity incorporated in the ribo-

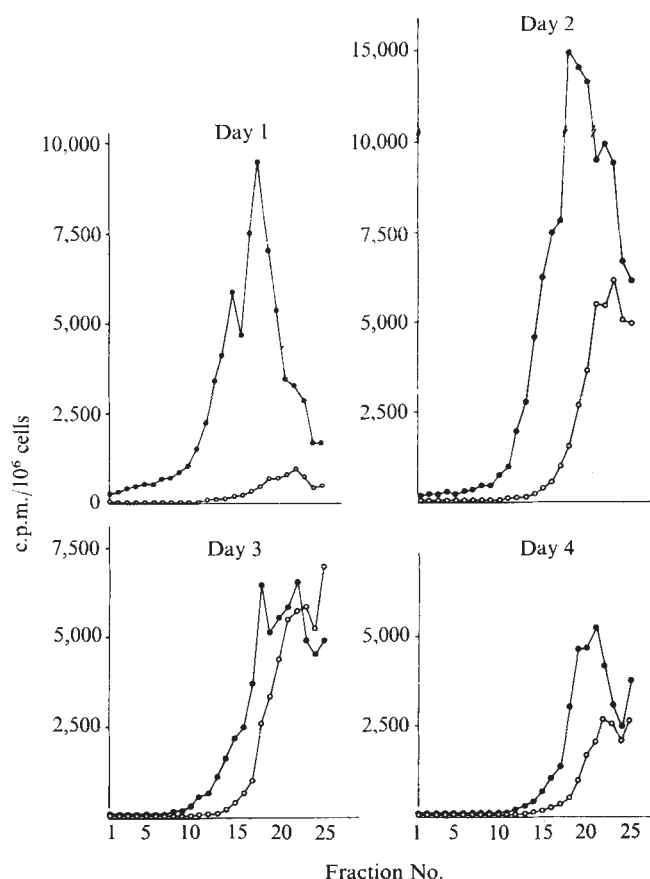


Fig. 1 Incorporation of ³H-uridine in ribosomes and polyribosomes each day after the 36th subcultivation of human fibroblasts carried with (●—●) and without (○—○) hydrocortisone. Cultures were labelled with 5 μ Ci ml⁻¹ ³H-uridine (specific activity 10 Ci mmol⁻¹) for 3 h. The cells were then scraped with a rubber policeman, washed twice in pH 8.6 buffer (0.01 M Tris, 0.14 M NaCl, 0.01 M MgCl₂) and treated for 10 min in the cold with 0.5% NP⁴⁰ in buffer. The cell suspensions were further treated with a Dounce homogeniser and examined under a microscope to see if nuclei were well separated from the cytoplasm. The cell suspensions were then centrifuged at 20,000g for 15 min and the supernatants were layered on a 7-52% sucrose gradient which was centrifuged at 25,000 r.p.m. for 3.5 h at 4°C in a SW 27 rotor. The gradients were collected in 25 fractions and to each fraction 10% TCA was added. The fractions were filtered through millipore filters which were digested in Instagel and the radioactivity counted in a liquid scintillation spectrometer. The bottom of the gradient is on the left side of the abscissa.

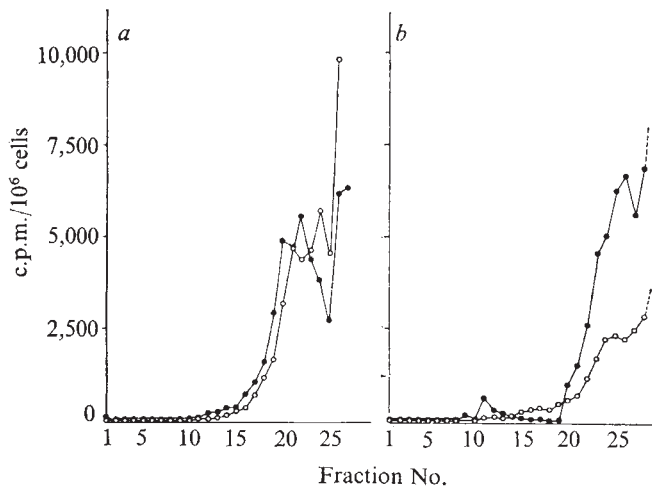


Fig. 2 Incorporation of ^3H -uridine into ribosomes during *a*, the first and *b*, the second passage after withdrawal of the hormone (○—○) in cells that were cultured with hydrocortisone (●—●) beyond the lifespan of the controls. Cells were labelled at the stationary phase and treated as described in Fig. 1. The bottom of the gradient is on the left side of the abscissa.

somal fraction had declined significantly in both groups. Ribosomal synthesis, however, was higher in the hydrocortisone-treated cells. No difference between the groups could be detected in the acid-soluble fractions, suggesting that the increased incorporation of uridine is not due to an increased penetration of the precursor into the cell.

Since cells cultured in hydrocortisone beyond the lifespan of the controls died within two passages when cultured in medium without the hormone, we decided to measure ^3H -uridine incorporation under these circumstances. Figure 2 shows that there was no difference in the incorporation of ^3H -uridine into ribosomes from both groups during the first passage after withdrawal of the hormone. A significant decrease, however, was found in the ribosomes synthesised after the second passage without the hormone. To be sure that this peak corresponds to ribosomes, we separated the ribosomal subunits on a 5–20% sucrose gradient (Fig. 3*a*) and then layered on a 7–52% sucrose

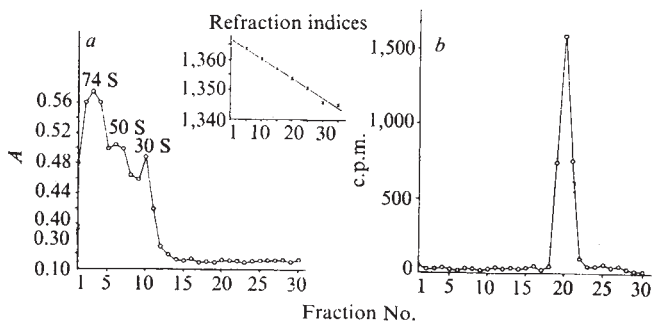


Fig. 3 *a*, Separation of ribosomal subunits on a 5–20% sucrose gradient in control human fibroblasts. Cells were labelled and the cytoplasmic fraction was prepared as described in Fig. 1 and then treated with 0.2% sodium deoxycolate in buffer at 4°C for 30 min. The suspension was then centrifuged for 2 h at 105,000g in a Beckman 40 rotor. The precipitate was resuspended in 1 ml pH 7.45 buffer (0.0011 M Na phosphate, 0.0004 M MgCl_2) and layered on a 5–20% sucrose gradient which was centrifuged for 2.5 h at 25,000 r.p.m. in a SW 27 rotor. The absorbance of the fractions collected from the gradient was measured at 260 nm. *b*, The fractions corresponding to the ribosome subunits were layered on a 7–52% sucrose gradient and the radioactivity was measured as described in Fig. 1. The bottom of the gradient is on the left side of the abscissa.

gradient (Fig. 3*b*) identical to the ones described in Fig. 1. All subunits were found around fraction 20.

Protein synthesis was also measured in hydrocortisone-treated cells and in the controls. At the 36th passage cultures from each group were pooled and subcultivated separately. Each day thereafter the cells were counted and labelled for 15 min with ^{14}C -labelled amino acids until the growth curves reached a plateau. Acid-soluble and insoluble radioactivity were measured. A significantly increased protein synthesis (Fig. 4) was found on the first

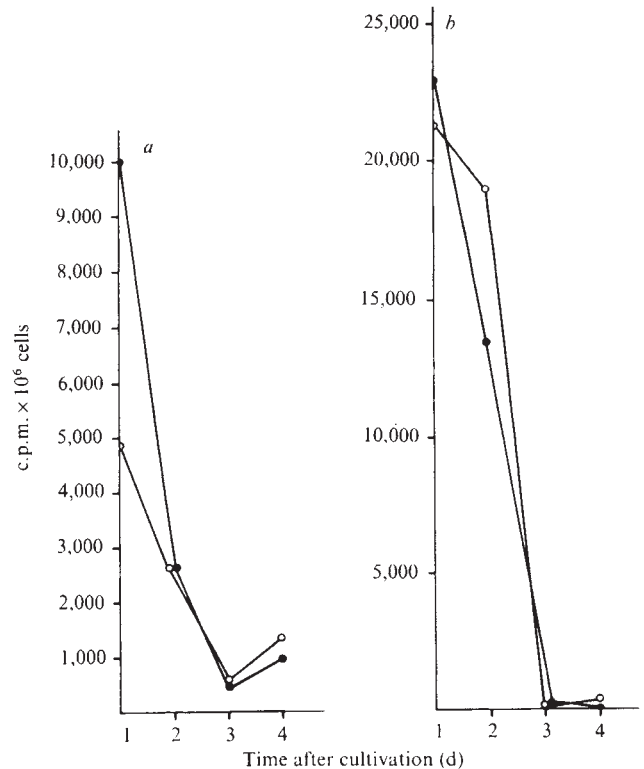


Fig. 4 Incorporation of ^{14}C -labelled amino acids into *a*, acid insoluble and *b*, acid soluble fractions each day after subcultivation of human fibroblasts carried in medium with (●—●) and without (○—○) the hormone. Cells were washed with phosphate-buffered saline (PBS) and then left for 15 min in PBS supplemented with $1\ \mu\text{Ci ml}^{-1}$ ^{14}C -labelled amino acids (Centre d'Energie Atomique, Saclay) (specific activity $0.1\ \text{Ci mmol}^{-1}$). The cells were scraped, washed three times in cold 10% TCA and the suspension was passed through millipore filters which were digested in Instagel. The supernatants were pooled and 0.5 ml was added to 10 ml Instagel to measure the radioactivity in a liquid scintillator.

day after subcultivation in the cells carried in hydrocortisone supplemented medium. When protein synthesis declined (due to cells reaching stationary phase) no difference could be seen in the two groups.

The results confirm previous findings that cortisone¹ and hydrocortisone² prolong the lifespan of human fibroblasts *in vitro*. It is interesting, however, that when hydrocortisone is withdrawn from cells which have grown beyond the lifespan of the controls, the cultures die within two passages. It has been suggested¹⁶ that cell senescence is due to an accumulation of errors at the level of protein synthesis and consequently an increased production of faulty molecules. If this is the case hydrocortisone does not prevent the accumulation of errors since the cells die when the hormone is withdrawn. Our data would be compatible with the error hypothesis if it is postulated that the hormone delays the deleterious effects of the presence of faulty

molecules by an overall stimulation of protein synthesis, by stimulation of ribosome synthesis.

A. MACIEIRA-COELHO
E. LORIA

*Institut de Cancérologie et d'Immunogénétique de
l'INSERM,
94800-Villejuif, France*

Received March 28; revised June 3, 1974.

- ¹ Macieira-Coelho, A., *Experientia*, **22**, 390 (1966).
- ² Cristofalo, V. J., in *Ageing in Cell and Tissue Culture* (edit. by Holeckova, E., and Cristofalo, V. J.), 83 (Plenum Press, New York, 1970).
- ³ Yuan, G. C., Chang, R. S., Little, J. B., and Cornil, G. J., *J. Gerontol.*, **22**, 174 (1967).
- ⁴ Macieira-Coelho, A., *Eur. J. clin. biol. Res.*, **17**, 925 (1972).
- ⁵ Levine, E. M., Becker, Y., Boone, C. W., and Eagle, H., *Proc. natn. Acad. Sci., U.S.A.*, **53**, 350 (1965).
- ⁶ Ward, G. A., and Plagemann, P. G. W., *J. cell. comp. Physiol.*, **73**, 213 (1969).
- ⁷ Stanners, C. P., and Becker, J., *J. cell. comp. Physiol.*, **77**, 31 (1971).
- ⁸ Becker, H., Stanners, C. P., and Kudlow, J. E., *J. cell. comp. Physiol.*, **77**, 43 (1971).
- ⁹ Emerson, C. P., *Nature new Biol.*, **232**, 101 (1971).
- ¹⁰ Hodgson, J. R., and Fisher, H. W., *J. Cell Biol.*, **49**, 945 (1971).
- ¹¹ Macieira-Coelho, A., Ponten, J., and Philipson, L., *Expl Cell Res.*, **42**, 673 (1966).
- ¹² Feigelson, M., Gross, P. R., and Feigelson, P., *Biochim. biophys. Acta*, **55**, 495 (1962).
- ¹³ Greengard, D., Smith, M. A., and Acs, G., *J. biol. Chem.*, **238**, 1548 (1963).
- ¹⁴ Jerwell, K. F., and Osnes, J. B., *Life Sci.*, **12**, 975 (1963).
- ¹⁵ Brouty-Boyé, D., Macieira-Coelho, A., Fizman, M. Y., and Gresser, I., *Int. J. Cancer*, **12**, 250 (1973).
- ¹⁶ Orgel, L. E., *Proc. natn. Acad. Sci., U.S.A.*, **49**, 517 (1963).
- ¹⁷ Winckelmans, D., Hill, M., and Errera, M., *Biochim. biophys. Acta*, **80**, 52 (1964).

Table 1 Effects of intravenous injection of B6AF1 anti-B10.D2 serum and rabbit complement on the survival of B10.D2 skin grafts in nude mice

Group no.	No. of recipients	Treatment	No. of grafts showing rejection	
			Complete	Patchy necrosis
1	6	+	4	2
2	5	+	0	0
3	3	+	0	0
4	4	—	0	4
5	4	—	2	2
6	4	—	0	0

* 0.5 ml intravenously on day 8

† 0.25 ml intravenously on day 8. Second injection of 0.25 ml 8 h later.

animals developed ascites and could be tapped every 1 to 2 weeks. The ascites fluid was pooled and tested with B10.D2 lymphoid cells by using the trypan blue dye exclusion method. Two different pools were used, both having an antibody titre of 1:512. Fresh frozen sera from rabbits were used as the source of complement.

The results are presented in Table 1. All mice which received injections of antiserum and rabbit complement showed necrotic changes of their grafts within 2 d after the injection (group 1). After 4 d four grafts were completely destroyed, while the other grafts showed extensive necrosis leaving only 40–50% of the epithelium intact. After the injection of antiserum alone no changes in the grafts were seen (group 2). If rabbit complement was inactivated by heating at 56° C for 45 min before its injection with antiserum, the grafts did not show signs of rejection (group 3). But in the control experiments where fresh frozen rabbit serum was given without the antiserum, grafts showed patchy necrosis after 24–48 h (group 4). This latter finding might be the result of the presence in rabbit serum of antibodies directed against mouse cells⁶. This was unlikely, since the rabbit serum was not toxic to mouse cells *in vitro*. Furthermore, it could still induce graft destruction after previous absorption with B10.D2 lymphoid cells (1 ml of packed cells to 5 ml of rabbit complement) for 10 min at 4° C (group 5). After its inactivation at 56° C for 45 min the rabbit serum did not cause destruction of the grafts (group 6). The deleterious effect of heat-labile factors in the rabbit serum on the skin grafts (groups 4 and 5) might be explained by the fact that the nude mice were able to form antibodies directed against B10.D2 antigens, which could induce acute destruction of the grafts after the addition of an efficient complement to the system. We, therefore, tried to demonstrate the presence of antibody activity directed against B10.D2 lymphoid cells in the sera of nude mice that carried a B10.D2 skin graft. The mice were bled on day 8 after transplantation, and the sera were tested in a trypan blue assay¹. Different dilutions of serum (0.1 ml) and of cell suspension (0.1 ml, 5×10^5 cells) were incubated with 0.1 ml of rabbit complement at 37° C for 30 min. Figure 1 shows that there was definite cytotoxic activity in these sera. C57BL6/Rij (H-2^b) lymphoid cells, serving as control cells, were not lysed by the serum. Several mice were tested separately, and all showed comparable antibody activity.

Three conclusions can be drawn from these results. First, the experiments show that skin grafts are sensitive to the cytotoxic action of alloantibody *in vivo* in a model where cell-mediated immunity is virtually absent. Second, it is clear that nude mice are capable of forming antibodies directed against transplantation antigens. This is in accordance with other studies where these mice were shown to

Rejection of skin grafts in the nude mouse

We recently reported that in (C57BL6/Rij $\times$ A/HeJ) F₁ (=B6AF1) mice, in which cellular immunity was temporarily suppressed by anti-lymphocyte serum, established B10.D2 skin allografts were hyperacutely rejected after the administration of specific alloantibody and rabbit complement¹. From this it was concluded that mouse skin allografts are sensitive to the cytotoxic action of alloantibody *in vivo* provided an effective species of complement is present at the same time.

Nude mice do not reject allogeneic or xenogeneic skin grafts. This phenomenon is usually explained by their inability to mount an effective cellular immune response^{2,3}. They, therefore, might serve as an ideal model for studying the effects of antibody on the fate of skin grafts. We now report preliminary results of experiments in which acute rejection of skin allografts by antibody was induced in nude mice.

B10.D2 (H-2^d) tail skin was grafted on to nude recipients by the procedure of Billingham and Medawar⁴. The nude trait was bred on to a B10.LP (H-2^b) background to produce a congenic line, according to the method described by Snell⁵. Mice of the second backcross generation were used. In all experiments donor and recipient were of the same sex. The fate of the grafts was followed by macroscopic inspection. Untreated nude mice carried their grafts until death without any signs of rejection. To induce acute rejection, nude mice in the experimental group received a single intravenous injection of 0.5 ml B6AF1 anti-B10.D2 serum (antiserum to H-2.31)¹ and two injections of 0.25 ml of rabbit complement, on day 8 after transplantation. The antiserum was prepared by injecting B6AF1 recipient mice at weekly intervals with a suspension of B10.D2 lymphoid cells in complete Freund's adjuvant. After five injections the

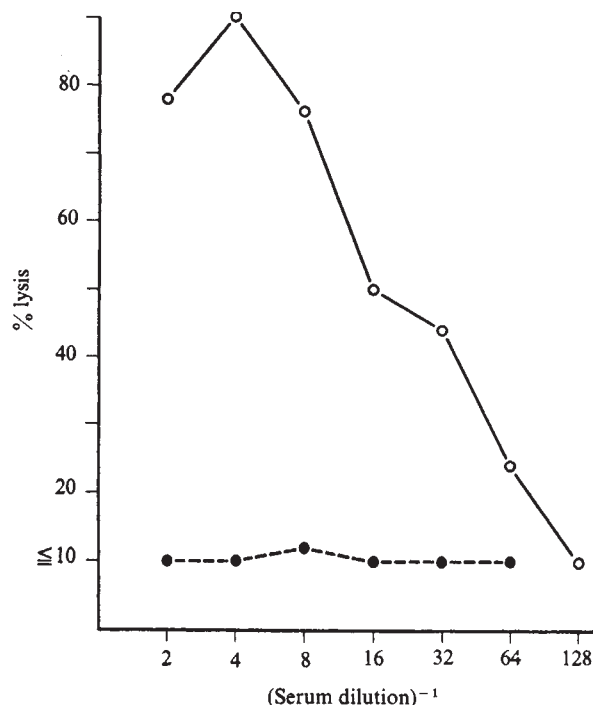


Fig. 1 Cytolysis of O, B10.D2 and ●, C57BL/6/Rij lymphoid cells by nude serum and rabbit complement. Mice were bled at day 8 after transplantation.

form antibodies against thymus-dependent antigens, for example, sheep red blood cells^{7,8}. Third, the results of our experiments suggest that the antibodies, which are formed after skin grafting, are able to destroy these grafts if an efficient species of complement is added to the system. The mouse native complement is very inefficient, at least in *in vitro* cytotoxicity systems⁹. This has been established not only in strains with a genetically determined deficiency of the C5 component of the complement system, but also in many other strains so far tested¹⁰. It probably holds true for the species as a whole. We found in our control experiments that fresh nude serum collected at day 8 after grafting, could only lyse B10.D2 cells after the addition of rabbit complement. This suggests that also in this strain endogenous complement is relatively inefficient *in vitro*. The indefinite survival of skin grafts in nude mice is probably not only caused by the insufficiency of the cellular immune response in these animals, but also by their lack of an efficient complement system.

This work was supported by grants from the Netherlands Foundation for Medical Research and from the Netherlands Kidney Foundation. We thank J. Koedam for technical assistance.

ROBERT A. P. KOENE
PAUL G. G. GERLAG
JAN J. JANSSEN
JACQ. F. H. HAGEMANN
PAUL G. A. B. WIJDEVELD

Department of Medicine, Division of Nephrology,
University of Nijmegen,
St Radboudziekenhuis,
Nijmegen, The Netherlands

Received May 17; revised June 7, 1974.

¹ Koene, R. A. P., Gerlag, P. G. G., Hagemann, J. F. H. M., van Haelst, U. J. G., and Wijdeveld, P. G. A. B., *J. Immunol.*, **111**, 520-526 (1973).

² De Sousa, M. A. B., Parrott, D. M. V., and Pantelouris, E. M., *Clin. exp. Immunol.*, **4**, 637-644 (1969).

- ³ Manning, D. D., Reed, N. D., and Shaffer, C. F., *J. exp. Med.*, **138**, 488-494 (1973).
⁴ Billingham, R. E., and Medawar, P. B., *J. exp. Biol.*, **28**, 385-402 (1951).
⁵ Snell, G. D., *J. natn. Cancer Inst.*, **21**, 843-877 (1958).
⁶ Koene, R. A. P., and McKenzie, I. F. C., *J. Immunol.*, **111**, 1894-1901 (1973).
⁷ Pantelouris, E. M., *Immunology*, **20**, 247-252 (1971).
⁸ Wortis, H. H., *Clin. exp. Immunol.*, **8**, 305-317 (1971).
⁹ Winn, H. J., *Ciba Foundation Symposium on Complement*, 133-154 (Churchill, London, 1965).
¹⁰ Rosenberg, L. T., and Tachibana, D. K., *J. Immunol.*, **89**, 861-867 (1962).

Spontaneous sister chromatid exchanges detected by a BUdR-labelling method

THERE is still controversy over the nature of sister chromatid exchanges detectable by autoradiography. Based on the relation between the number of exchanges and dose of tritium incorporated into DNA, Gibson and Prescott¹ have concluded that all exchanges are radiation-induced and that none occurs spontaneously. But such an approach has an apparent technical drawback in that the declining of sensitivity of emulsion to tritium as well as latent image fading during long autoradiographic exposure provides a definite lower limit in the number of grains formed at a given tritium dose, and hampers greatly the detection of exchanges at very low dose of incorporated tritium. Another

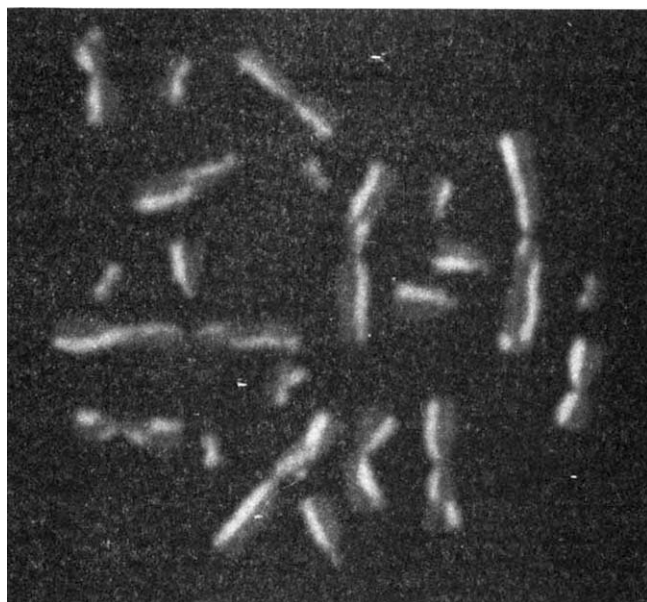


Fig. 1 Fluorescence photomicrograph of metaphase chromosomes stained with acridine orange. This cell had previously been labelled with $1.0 \mu\text{g ml}^{-1}$ BUdR for 26 h and fixed according to the air-drying method following 1 h Colcemid treatment. $\times 1,600$. The cell had an exceptionally large number of exchange sites (11 exchanges per cell). The brightly stained chromatid was probably composed of DNA with only one strand substituted with BUdR, the duly stained one being composed of DNA with both strands substituted with BUdR. Demarcation was also possible between a chromatid containing a non-BUdR substituted DNA duplex and its sister chromatid composed of a duplex with only one strand substituted with BUdR. These chromatids could be obtained by pulse-labelling cells with BUdR and then growing them for another cell cycle in the absence of BUdR before fixation. Such labelling procedures were not employed in this study so as to avoid a risk of exposure of BUdR-labelled cells to visible light which would cause breaks in BU-substituted DNA, and hence, would affect the frequency of sister chromatid exchanges.

difficulty may arise from the fact that the exchange frequency reaches a saturation level at a very low dose and within a very narrow dose range, so that it is difficult to determine whether a dose response curve rises linearly with or as the square of dose. Extrapolation of the curve through the zero point can be misleading. To detect sister chromatid exchanges, I have, following the original work of Latt³, used a new method involving labelling of chromosomes with BUdR followed by acridine orange fluorescence staining, and have obtained results that suggest strongly the existence of spontaneous sister chromatid exchanges.

A pseudodiploid Chinese hamster cell line, D-6, was grown in total darkness in culture medium² containing BUdR at concentrations ranging from $0.1 \mu\text{g ml}^{-1}$ to $40 \mu\text{g ml}^{-1}$ for two cell cycles and fixed for chromosome preparation. Slides were stained with 0.125 mg ml^{-1} acridine

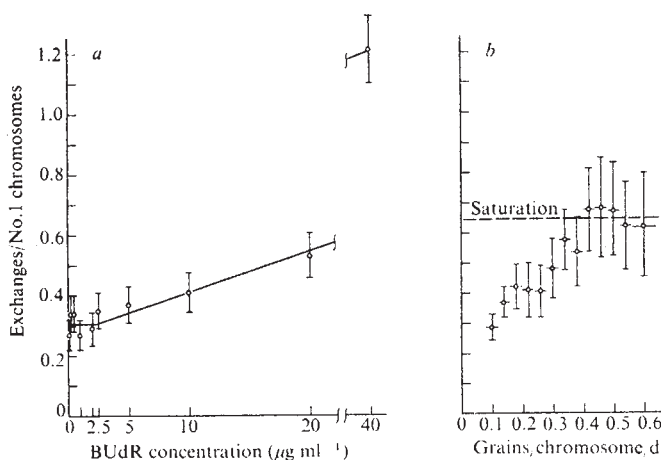


Fig. 2 *a*, Sister chromatid exchanges scored in No. 1 chromosomes of BUdR-labelled cells. Each point represents 100 chromosomes scored. Range bars indicate s.e.m. *b*, sister chromatid exchanges scored in No. 1 chromosomes of cells which had been labelled with ^3H -thymidine ($0.005 \mu\text{Ci ml}^{-1}$ and $0.01 \mu\text{Ci ml}^{-1}$, specific activity $12.1 \text{ Ci mmol}^{-1}$) for 4 h and fixed in the second postlabelling mitosis. Slides were coated with emulsion (Sakura NR-M2) and exposed for 132 d. The number of grains as well as of exchange sites were scored in 755 no. 1 chromosomes. Range bars represent s.e.m. and the horizontal line for each point indicates the range of grain number used to determine each point. The broken line marked 'saturation' indicates the level of the maximum exchange frequency at high doses of incorporated tritium.

orange in phosphate buffer, pH 6, for 5 min, washed for 15 min with and mounted in the same buffer. Sister chromatids were demarcated clearly, one being stained brightly and the other dull (Fig. 1). This method allowed unequivocal detection of exchanges of even very short segments of sister chromatids which would probably escape detection by the conventional autoradiographic method. The mechanisms underlying this differential staining remains to be elucidated. Fluorescence of acridine orange might also be affected by a quenching effect of BUdR as reported for 3325 Hoechst fluorescence³.

In Fig. 2*a* is plotted against BUdR concentrations the frequency of sister chromatid exchanges in No. 1 chromosomes. The frequency at $0.1 \mu\text{g ml}^{-1}$ was about 0.3 exchanges per chromosome and it remained constant up to $2.0 \mu\text{g ml}^{-1}$. With further increase in the concentration, the frequency started to rise and reached a level about four times of the constant level at $40 \mu\text{g ml}^{-1}$. Since BUdR labelling at concentrations lower than $0.1 \mu\text{g ml}^{-1}$ did not permit unequivocal demarcation of sister chromatids, it is uncertain whether the constant portion of the curve indicates a spontaneous exchange frequency or whether it

would fall abruptly to zero at a lower concentration. Results obtained with the conventional autoradiographic method are given in Fig. 2*b* for comparison. A dose-dependent increase in the exchange frequency was observed within the dose range 0.1 grains to 0.4 grains per chromosome per d confirming previous workers' results¹. The fate of the frequency curve at lower tritium doses is unknown; however a simple extension of the declining curve does not reach zero but meets the ordinate at a point between 0.2 and 0.3 exchanges per chromosome, which approximates to the value of the constant portion of the frequency curve obtained in BUdR-labelled cells (Fig. 2*a*).

In Fig. 3 the response to BUdR of sister chromatid exchanges and chromatid breaks are compared. It is noticeable that frequencies of both events stayed at constant levels between $0.1 \mu\text{g ml}^{-1}$ and $1.0 \mu\text{g ml}^{-1}$, and that the constant level of the frequency of breaks was exactly the same as that of non-BUdR treated controls. These results suggest strongly that the constant portion of the frequency curve of sister chromatid exchanges might also represent a spontaneous exchange frequency. Other possibilities, however, cannot yet be ruled out completely; for example (1) the genesis of sister chromatid exchanges might be completely different from that of chromatid breaks, so that the comparison of the two events in terms of their responses to BUdR might be meaningless; (2) there may be an upper limit for the BU substitution sites in DNA at low BUdR concentration⁴, and such a limited level would be easily saturated, leaving the frequency of BUdR-induced sister chromatid exchanges constant.

Since the exchange frequency observed between $0.1 \mu\text{g ml}^{-1}$ and $1.0 \mu\text{g ml}^{-1}$ (2.3 exchanges per cell) is the sum of exchanges formed during two successive cell cycles, the average frequency per cell generation is 1.15. If this frequency is ascribed to spontaneous exchanges, the rate may seem to be rather high considering that strand exchange

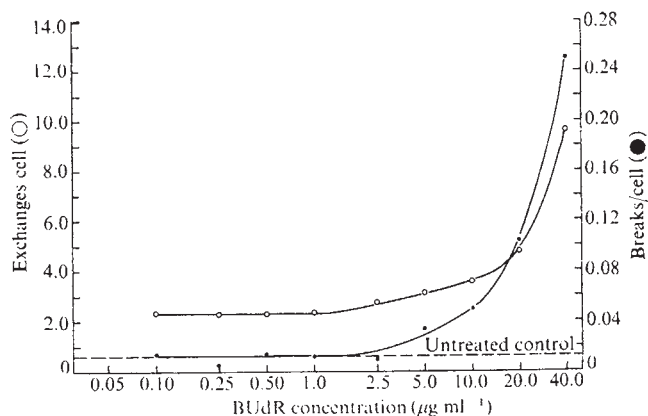


Fig. 3 The number of sister chromatid exchanges per cell (○) is shown together with the frequency of breaks per cell (●). Each point on the curve for the exchange frequency was determined based on 50 acridine orange-stained cells. Chromatid-type aberrations were scored in cells stained with Giemsa. Each point on the frequency curve represents 200 cells scored, and is plotted in a 50-fold larger scale than that for sister chromatid exchanges. Chromosome-type aberrations, both the 'derived-type' aberrations and those induced in G1, were omitted from the score. Thus, the value presented in this figure is an underestimation for breaks per cell per generation. Chromatid gaps were detected at almost the same frequency as that of chromatid deletions but they were omitted from this figure. The broken line indicates the frequency of breaks observed in the non-BUdR treated control. Whereas sister chromatid exchanges occurred randomly in every chromosomal complement at seemingly nonspecific sites, chromatid breaks as well as chromatid gaps were detected only in a few specific chromosomes such as No. 1 and X. The break points were mostly confined to constitutive heterochromatin regions.

is an error-prone process⁵. But data showing that spontaneous DNA damage probably occurs to a significant extent in the normal conditions are accumulating. Lindahl and Nyberg⁶ estimate that 2,000–10,000 purines would be lost from mammalian DNA in each cell generation. These apurinic sites seem to be repaired continuously by a maintenance system similar to the excision repair mechanism⁷. Spontaneous unscheduled DNA synthesis detectable in mammalian cells may be regarded as an outcome of such an error-correcting mechanism⁸. Although exact molecular processes leading to the formation of sister chromatid exchanges remain to be elucidated, they seem to be intimately related to mechanism of DNA repair⁵. It is uncertain yet whether sister chromatid exchanges are the outcome of a normal repair process or of errors in repair mechanisms. Whichever they may be, it is possible that one out of several thousand damaged sites in DNA would fail to reconstitute but provoke strand exchanges. In the same context, chromosomal breaks probably result from misrepair of DNA damage. A spontaneous frequency of breaks per cell per cell generation was about 0.012. This would mean that sister chromatid exchanges occur at a frequency about 100 times of that of chromatid breaks in the normal conditions. This ratio seems to be variable when both events are induced by exogenous agents (Fig. 3).

The observation that BUdR at high concentrations readily induced sister chromatid exchanges may be relevant to the findings that BU enhances the recombination frequency in λ phage and that a recombinational repair process is involved in BU-induced mutagenesis⁹.

HATAO KATO

Department of Cytogenetics,
National Institute of Genetics,
Mishima, 411 Japan

Received April 29, 1974.

¹ Gibson, D. A., and Prescott, D. M., *Expl Cell Res.*, **74**, 397–402 (1972).

² Kato, H., *Expl Cell Res.*, **82**, 383–390 (1973); *Expl Cell Res.*, **83**, 55–62 (1974); *Nature*, **249**, 552 (1974); *Expl Cell Res.* (in the press).

³ Latt, S. A., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3395–3399 (1973).

⁴ Simon, E. H., *Expl Cell Res.*, Suppl. 9, 263–269 (1963).

⁵ Witkin, E. M., *A. Rev. Genet.*, **3**, 525–552 (1969).

⁶ Lindahl, T., and Nyberg, B., *Biochemistry*, **11**, 3610–3618 (1972).

⁷ Verly, W. G., Paquette, Y., and Thibodeau, L., *Nature new Biol.*, **244**, 67–69 (1973).

⁸ Djordjevic, B., Evans, R. G., Perez, A. G., and Weill, M. K., *Nature*, **224**, 803–804 (1969).

⁹ Pietrzykowska, I., *Mutation Res.*, **19**, 1–9 (1973).

Non-random loss of human markers from man–mouse somatic cell hybrids

THE preferential loss of human chromosomes from hybrids between human and mouse somatic cells¹ is being used in the assignment of human gene loci to chromosomes². When the experimental design does not select for the gene product under study, the assignment often assumes that the loss of chromosomes is random. For example, hybrid clones derived from mouse cells and SV40-transformed human skin fibroblasts lose the SV40-induced T antigen only when all but a few human chromosomes have been eliminated³. If the pattern of chromosome loss is random, these results could indicate that SV40 DNA has integrated into the DNA of several chromosomes. They might be attributable, however, to the scheduled elimination of a single pertinent human chromosome relatively late in the evolution of each

clone, a possibility supported by the finding of a preferential retention of C-7 in hybrids derived from SV40-transformed human cells and a concordance of this chromosome with the presence of SV40 and T antigen⁴. Moreover, if the loss of chromosomes were directed, joint segregation of two chromosomes might occur, making it difficult to decide which of the two specified the pertinent gene product being lost simultaneously.

We have studied the pattern of loss of human enzyme and chromosome markers from hybrid clones by means of single observations of many clones, as well as observation of the evolution of individual clones, which is a more sensitive measure of directed loss. Our findings suggest that in our system the elimination of human chromosomes is indeed not random.

As the murine parent, mouse fibroblasts (3T3) lacking thymidine kinase (EC 2.7.1.21, TK) were chosen because they can be eliminated by selective medium and because

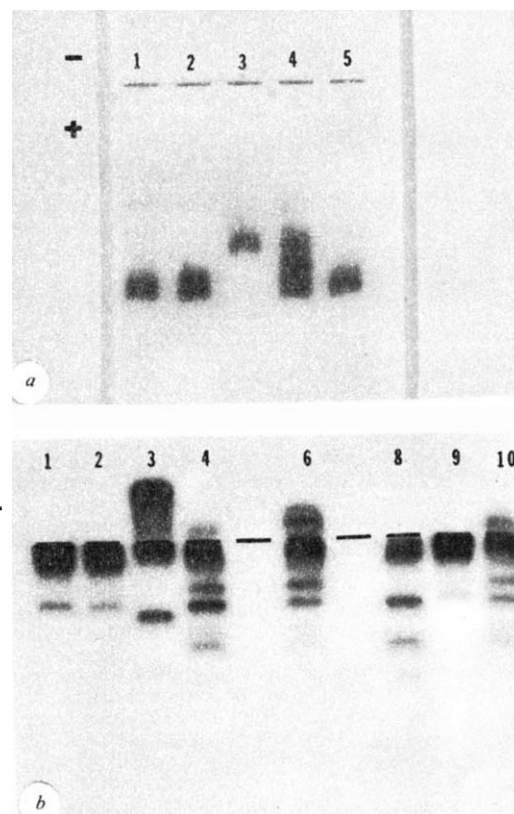


Fig. 1 Enzyme pattern of 3T3, human fibroblasts and hybrids. Electrophoresis of cell extracts⁹ was performed on starch gels¹⁰ which were stained for G6PD¹¹ or LDH¹². *a*, G6PD zymogram: channel 1, 3T3; 2 and 5, hybrids without human G6PD; 3, normal human fibroblasts; 4, hybrid with human and mouse G6PD and heteropolymer. *b*, LDH zymogram: channels 1 and 2, hybrids without human LDH; 3, normal human fibroblasts; 4, 6, and 10, hybrids showing heteropolymers of human and mouse LDH A subunits and LDH B subunits presumably of human origin; 8, hybrid with LDH B subunits and no human LDH A subunits; 9, 3T3. The mouse fibroblasts have a single band of strong activity which migrates with mouse LDH 5 and therefore consists solely of LDH A subunits. The LDH B subunits of mouse and man share the same electrophoretic mobility, so that a band anodal to that of 3T3 indicates the presence of either mouse or human LDH B structural genes. Since the 3T3 parent has no B subunits, we have attributed any novel bands anodal to that of 3T3 to the presence of a human gene influencing this phenotype. Isozymes migrating cathodally to that of 3T3 were ascribed to the presence of the human LDH A subunit. Combinations of novel bands anodal and cathodal to that of 3T3 were interpreted as the simultaneous presence of human LDH subunits A and B.

Table 1 Analysis of a hybrid clone maintained in HAT medium

Subculture no.	LDH B	LDH A	G6PD
3	+	+	—
8	+	+	—
10	+	+	—
12	+	+	—
14	+	+	—
16	+	—	—

This table illustrates the method of analysis applied to each clone. Cell extracts at various subcultures were assayed for human enzymes until the clone showed only one marker.

the karyotype, containing almost exclusively acrocentric-telocentric chromosomes, cannot be confused with the human karyotype. We used a clone with only a rare cell containing a banded chromosome. To ensure a normal human parental karyotype, we used unstimulated leukocytes from a normal human male which were prepared⁵ and washed twice with saline. Fibroblasts (2×10^6) and leukocytes (2×10^7) were fused in suspension using β -propiolactone-inactivated Sendai virus⁶ and plated in HAT selective medium⁷ with amethopterin (10^{-7} M, Lederle) substituted for aminopterin.

Forty HAT-resistant clones were isolated with cloning cylinders from 24 dishes between 13 and 33 d after fusion. Multiple clones from a single dish were transferred individually 13 d after fusion. As they were all about the same size, they presumably originated from different heterokaryons. When these clones became confluent, half of each was plated into a dish containing HAT and the other half into a dish containing a non-selective medium, HT (HAT without amethopterin). Thus we compared the effect of amethopterin on the pattern of marker elimination. At each subsequent subculture, one-fifth of the cells was used to continue the line and four-fifths were divided between two dishes, one for enzyme electrophoresis and one for

chromosome preparations. Lactate dehydrogenase subunit A (LDH A) and subunit (LDH B) (both EC 1. 1. 1. 27) and glucose-6-phosphate dehydrogenase (G6PD, EC 1. 1. 1. 49) were chosen as enzyme markers because mouse and human forms could be distinguished, because each is specified by a different chromosome⁸ and because they could be analysed simultaneously.

The characteristic G6PD patterns seen in our hybrids are shown in Fig. 1a. Only patterns with the G6PD heteropolymer were scored as positive for human G6PD. Some of the LDH isozyme patterns observed are presented in Fig. 1b.

Thirty of the HAT-resistant colonies survived transfer and showed human isozymes and/or human marker chromosomes. Each clone was analysed at various stages of its evolution until it had only one of the three enzyme markers (Table 1). Table 2 gives the frequency of the human enzymes in all hybrid clones analysed, including serial determinations of each during clonal evolution. The distribution of the frequencies indicates that markers were not lost randomly.

The predominance of LDH B reflects the timing of its elimination from individual hybrid clones (Fig. 2a and b). Since amethopterin in the medium did not affect the incidence of the human LDH subunits and only slightly influenced the retention of human G6PD (Table 2), our observations from the clonal sublines maintained in the two media have been pooled. The first enzyme lost was human G6PD in 17 of the 26 clones analysed. No clone lost human LDH B first (Fig. 2a). The last of the three human enzyme markers lost was LDH B in 22 out of 27 clones analysed (Fig. 2b). The number of clones either losing G6PD first or retaining LDH B until last is significantly different from the numbers expected if the loss were random ($P < 0.01$). Thus a distinct pattern of enzyme elimination was observed, characterised by the relatively early loss of human G6PD and the retention of LDH B.

Table 2 Frequency of human enzyme markers in hybrid clones

Medium	LDH B	Enzyme LDH A	G6PD
HAT	88% (80)*	68% (81)	38% (69)
HT	86% (109)	62% (107)	27% (106)
Total	87% (189)	64% (188)	31% (175)

*Number of different clonal extracts analysed for the presence of the pertinent marker. The extracts came from a total of 30 clones assayed at various times during their propagation and no duplicates are included.

To test whether our analysis had been influenced by a differential sensitivity of the assay for the three enzymes, we performed reconstruction experiments with parent cell lines. After starch gel electrophoresis, the distribution of LDH A and B subunits in extracts of human leukocytes is approximately equal. To determine the relative sensitivity of the electrophoretic assay, we used human skin fibroblasts rather than leukocytes, as the former seemed more closely to simulate the intracellular environment in the hybrid. Various proportions of fibroblasts were mixed with 3T3 cells and the extracts were subjected to electrophoresis. In our gel system human fibroblasts have predominantly LDH A subunits. Both LDH A and G6PD were equally detectable when as little as 5% of the mixture was human extract. Since the LDH B was slightly less visible than the other two marker enzymes in these preparations, the observed pattern of enzyme loss cannot be explained on the basis of differential sensitivity of the assay.

The early loss of G6PD is not surprising, since only one human X chromosome is present in the hybrid and since our data indicate that this chromosome provided no great selective advantage in HAT medium. Nevertheless, the

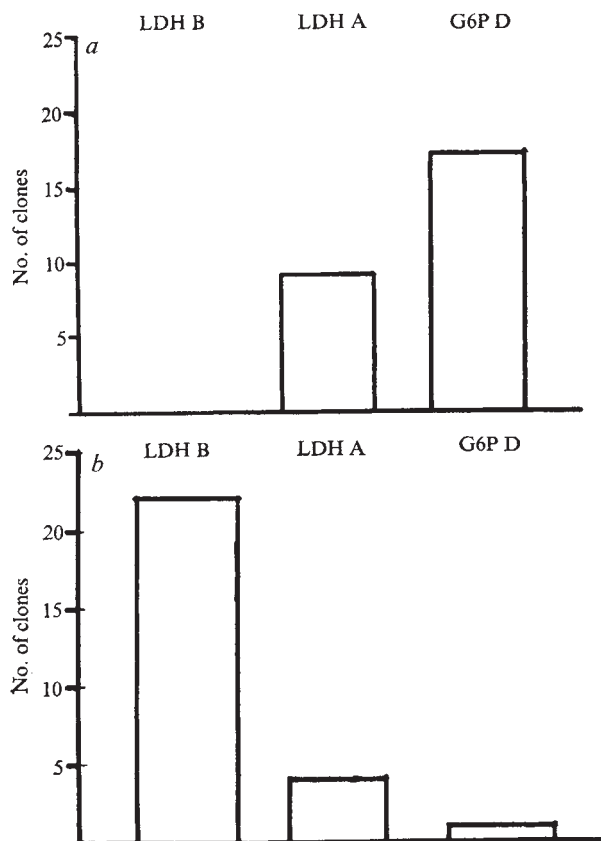


Fig. 2 Pattern of marker loss determined by serial observations of individual clones. *a*, Number of clones losing each of the enzyme markers first; *b*, number of clones losing each of the enzyme markers last.

preferential retention of human LDH B compared with LDH A cannot be explained on the basis of chromosome dose and is evidence of a directed pattern of chromosome loss.

The hybrid clones were analysed for the presence of human chromosomes 1, 3, 6, 7, 9, 11, 12, and Y because their distinctive quinacrine mustard (QM) fluorescence patterns¹³ facilitated discrimination from rearranged mouse chromosomes. The 3T3 clone used as mouse parent contained an occasional submetacentric chromosome at the time of fusion and these did not increase significantly. Moreover, no mouse chromosomes had fluorescent patterns that could be confused with the human chromosome markers. The karyotype of the human parent was characterised by intensely fluorescent centromeres of chromosome 3 and the terminal long arm of the Y. The 52 hybrid karyotypes analysed included 1 to 4 cells from each of 16 clones. Most hybrid cells had two sets of 3T3 chromosomes.

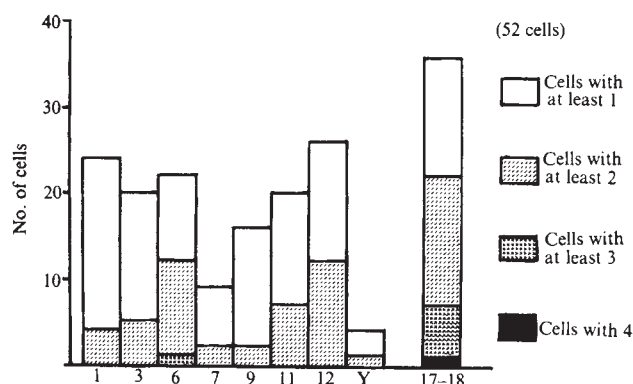


Fig. 3 Frequency of human chromosome markers in hybrid cells.

Because it is difficult to distinguish invariably chromosome E17 from E18, we have referred to a submetacentric E group chromosome as 17-18. However, we believe the chromosome is E17, since the thymidine kinase it specifies is essential to the survival of the hybrid cell.

Except for chromosome 17, the incidence of human chromosome markers did not differ significantly in HAT and non-selective medium. Therefore, we have combined observations of clonal sublines maintained in the two media for the analysis of the frequency of the various human chromosome markers (Fig. 3). The least frequently observed chromosome was the Y, possibly because the parent line contained only one. The most frequent was the chromosome specifying thymidine kinase¹⁴ as a result of its selective retention by the culture conditions. Moreover, we noted significant variation ($P < 0.01$) in the frequency of the autosomes not obviously favoured or disfavoured by the experimental conditions. Therefore, the distribution of chromosomes in the hybrid cells is not that expected if all chromosomes had an equal chance of inclusion in the hybrid genome. In addition, the presence of human LDH subunits, together with the prevalence of chromosome C-11 and C-12 in these hybrids, supports the assignment of LDH A and LDH B to these two chromosomes^{15,16}.

Data from studies of other hybrids suggest to us further evidence of non-random human marker loss. None of the nine clones derived by Santachiara *et al.*¹⁷ from fusion of 3T3 with human leukocytes of female origin had human G6PD, while four had human LDH A and seven had human LDH B. Allderice *et al.*¹⁸ studied the karyotype of man-mouse hybrid clones where selection favoured retention of the human X. Of the chromosome markers used by us and Allderice *et al.*, C-12 was second in incidence

only to the X, while C-11 was also frequently observed. Further evidence of preferential inclusion of chromosomes specifying LDH in the hybrid is that a disproportionate number of autosomal linkage assignments made on the basis of hybrids involved the joint segregation of the pertinent marker with A or B subunits of LDH.

The basis for the directed loss remains obscure, but may be related to the nature of the gene products specified by the chromosomes preferentially lost. In this case one might predict that the pattern of elimination could vary with the parental cells and culture conditions used.

This work was supported by grants from the National Institutes of Health.

ROBERT A. NORUM

BARBARA RUBEN MIGEON*

Department of Pediatrics,
Johns Hopkins University School of Medicine,
Baltimore, Maryland 21205

Received December 18, 1973; revised January 21, 1974.

*To whom reprint requests should be addressed.

- Weiss, M. C., and Green, H., *Proc. natn. Acad. Sci. U.S.A.*, **58**, 1104 (1967).
- Ruddle, F. H., *Nature*, **242**, 165 (1973).
- Weiss, M. C., *Proc. natn. Acad. Sci. U.S.A.*, **66**, 79 (1970).
- Croce, C. M., Girardi, A. J., and Koprowski, H., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3617 (1973).
- Bodmer, W., Tripp, M., and Bodmer, J., *Histocompatibility testing 1967* (edit. by Curtini, E. S., Mattiuz, P. L., and Tosi, R. M.), 341 (Williams and Wilkins, Baltimore, 1967).
- Miggiano, V., Nabholz, M., and Bodmer, W., *Wistar Inst. Symp. Monog.*, No. 9 (edit. by Defendi, V.), 61 (Wistar Institute Press, Philadelphia, 1969).
- Szybalski, W., Szybalska, E., and Ragni, G., *Natn. Cancer Inst. Monog.*, **7**, 75 (1962).
- Nabholz, M., Miggiano, V., and Bodmer, W., *Nature*, **223**, 358 (1969).
- Migeon, B. R., Corsaro, C. M., and Norum, R. A., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 937 (1974).
- Porter, I. H., Boyer, S. H., Watson-Williams, E. S., Adam, A., Szeinberg, A., and Siniscalco, M., *Lancet*, **i**, 895 (1964).
- Boyer, S. H., Porter, I. H., and Weilbaecher, R. G., *Proc. natn. Acad. Sci. U.S.A.*, **48**, 1868 (1962).
- Migeon, B. R., *Biochem. Genet.*, **1**, 305 (1968).
- Caspersson, T., Lomakka, G., and Zech, L., *Hereditas*, **67**, 89 (1971).
- Miller, O. J., Allderice, P. W., Miller, D. A., Breg, W. R., and Migeon, B. R., *Science*, **173**, 244 (1971).
- Boone, C., Chen, T. R., and Ruddle, F. H., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 510 (1972).
- Chen, T. R., McMorris, F. A., Creagan, R., Ricciuti, F., Tischfield, J., and Ruddle, F., *Am. J. human Genet.*, **25**, 200 (1973).
- Santachiara, A. S., Nabholz, M., Miggiano, V., Darlington, A. J., and Bodmer, W., *Nature*, **227**, 248 (1970).
- Allderice, P. W., Miller, O. J., Pearson, P. L., Klein, G., and Harris, H., *J. Cell Sci.*, **12**, 809 (1973).

Isolation and *in vitro* translation of soybean leghaemoglobin mRNA

LEGAEMOGLOBIN (Lb), a myoglobin-like protein, is found in large quantities in the root nodules which result from the symbiotic association between the bacterium *Rhizobium* and plants of the family Leguminosae. The probable function of Lb is the regulation of intranodule oxygen tension¹ and its presence is correlated with rhizobial nitrogenase². The haem prosthetic group of Lb is of bacterial origin³ and the plant contains the genetic determinants for the apoprotein^{4,5}. Soybean Lb consists of two major components of different electrophoretic mobility⁶. The faster component (LbF) is composed of 139 amino acids, has a molecular weight of 15,600 and contains an amino terminal glycine residue, while the slower component (LbS) is com-

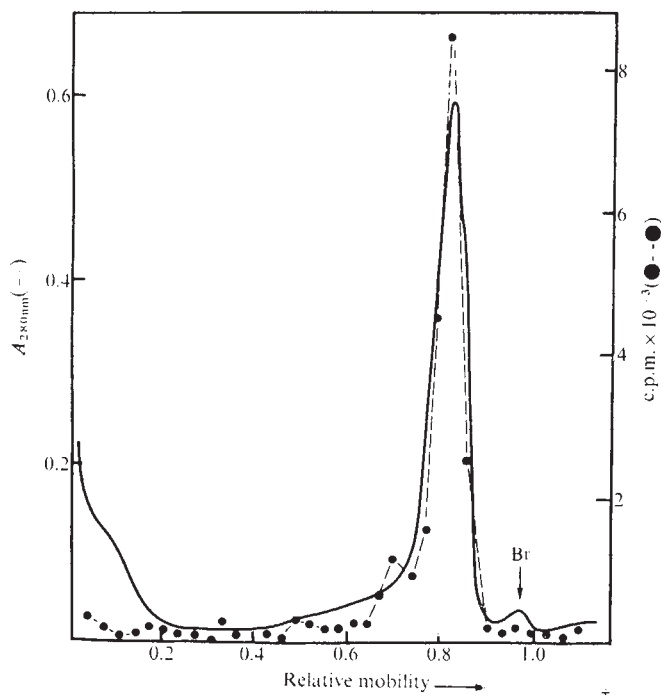


Fig. 1 SDS gel electrophoresis of the immunoprecipitated product from *in vitro* protein synthesis with nodule polysomes. Polysomes were isolated from nodules by homogenising the tissue in high ionic strength buffer (150 mM Tris-acetate; 20 mM KCl; 5 mM Mg acetate; 200 mM sucrose; 5 mM mercaptoethanol; 0.4% Nonidet P-40 detergent, pH 8.5). The polysomes were centrifuged through a 2 ml sucrose cushion (1.5 M sucrose; 50 mM Tris-acetate; 20 mM KCl; 5 mM Mg acetate, pH 8.5), for 2 h at 105,000g, and resuspended in 10 mM Tris-HCl; 20 mM KCl; 1 mM Mg acetate and 20% glycerol. 4–8 A_{260} units of polysomes were incubated with the standard amino acid incorporation system in 400 μ l consisting of: 80 μ l of postribosomal wheat supernatant; 45 mM KCl; 3.5 mM Mg acetate; 1 mM ATP; 25 μ M GTP; 8 mM creatine phosphate; 16 μ g creatine phosphate kinase; 25 mM Tris-acetate, pH 8.0; 2 mM dithiothreitol; 2 μ Ci 4,5- 3 H-L-leucine (specific activity 64 Ci mmol $^{-1}$). After 60 min of incubation at 30° C carrier antigen was added (2 μ g each of LbF and LbS) and ribosomes were removed by centrifugation. The postribosomal supernatant was reacted with anti-Lb serum for 60 min at 30° C. The immunoprecipitate was washed three times with phosphate-buffered saline, solubilised in SDS buffer and subjected to electrophoresis in 10% SDS gels⁹. Gels were cut into 2 mm slices and dissolved in 0.8 ml of 30% H₂O₂ at 55° C and counted in PCS (Amersham, Searle). The absorbance at 280 nm was determined on an identical gel run with Lb as a marker (50 μ g each of LbS and LbF).

posed of 143 amino acids, has a molecular weight of 15,900 and contains an amino terminal valine residue. Because of these differences LbF and LbS are presumed to be different gene products⁷. Thus they are useful markers in studies on the transfer of plant genes involved in symbiosis to other plants.

In an attempt to elucidate the interaction between plant and bacteria in the expression of Lb genes we have isolated a poly(A)-containing 9S–12S mRNA from polysomes of soybean root nodules and translated it in a wheat embryo cell-free system. This mRNA can initiate protein synthesis on 80S wheat ribosomes and release completed protein chains which react with monospecific antibodies to Lb. Translation of Lb mRNA is not dependent on the presence of haem or any other factor from soybean root nodules.

Rhizobium japonicum strain 61A76 (supplied by Dr Joe Burton, Nitragin Co., Milwaukee) was grown in a liquid culture medium composed of (g l $^{-1}$): K₂HPO₄, 0.5; MgSO₄·7H₂O, 0.2; NaCl, 0.1; yeast extract, 1.0; mannitol 10.0 and adjusted to pH 6.6. Bacteria were grown at 27° C with constant shaking and subcultured every 7 d. Soybean

(*Glycine max*, var Kanrich), was grown on Vermiculite in a controlled environment room under a 12 h photoperiod at 27° C and a night temperature of 21° C, and provided with a nitrogen-free nutrient solution³. Seeds were inoculated with a suspension of rhizobia in 5% sucrose before sowing and root nodules were collected 3–4 weeks later. The nodules were frozen immediately in liquid nitrogen and those 2–4 mm in diameter were used, except where mentioned otherwise.

Polysomes were prepared for translation experiments as outlined in the legend to Fig. 1. To test their capacity for protein synthesis, polysomes were incubated in a cell-free system containing postribosomal supernatant from wheat embryos^{9–11}. Under these conditions polysomes primarily complete and release nascent protein chains (our unpublished data). The radioactive product from this system reacted with anti-Lb serum obtained from rabbits immunised with electrophoretically homogeneous Lb. Since the antiserum was monospecific and the radioactive antigen (after solubilisation from the antigen-antibody complex) migrated with

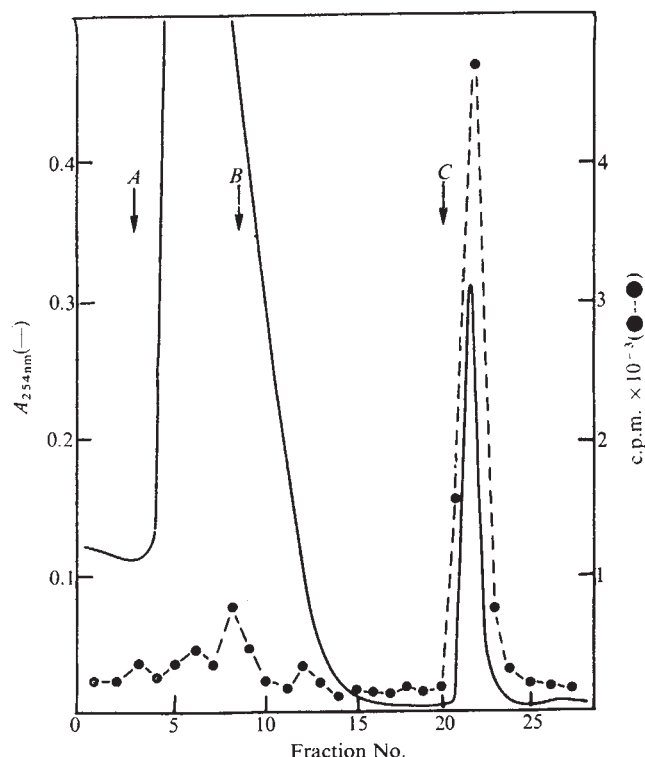


Fig. 2 Isolation of poly(A)-containing mRNA by oligo(dT)-cellulose chromatography. Polysomes, prepared as for Fig. 1, were suspended in buffer (0.1 M Tris-acetate; 0.1 M NaCl; 2 mM Na EDTA, pH 9.0) and made 1% with respect to SDS at room temperature. Total polysomal RNA was extracted and chromatographed as described by Aviv and Leder¹². The sample was applied at room temperature in SDS buffer (A). SDS was removed in the wash buffer (B). Poly(A)-containing RNA was eluted with 0.01 M Tris-acetate, pH 7.4 (C). Absorbance at 254 nm was monitored with a Beckman DB spectrophotometer. An aliquot of each fraction was incubated with 5 nCi of 3 H-poly(U) in a final volume of 0.5 ml containing 0.2 M NaCl; 5 mM Mg acetate; 10 mM Tris-acetate, pH 7.4. Hybridisation was allowed to proceed for 15 min at 25° C, after which each fraction was treated with 20 μ l of pancreatic RNase (20 μ g ml $^{-1}$) for 30 min at 25° C. The reaction was terminated by adding cold 5% trichloroacetic acid (TCA). Carrier RNA was then added and the precipitates collected on Whatman GF/C filters, washed with cold 5% TCA, dried, and their radioactivity measured. No 3 H-poly(U) binding was observed with controls which consisted of equivalent amounts of wheat ribosomes and/or wheat tRNA. The fractions which eluted with buffer (C) contained most of the poly(A)-containing RNA, were pooled and made 2% with respect to potassium acetate, pH 5.5. They were then precipitated with 2.5 volumes of ethanol at –20° C.

Table 1 Translation of root nodule RNA fractions and the immunoprecipitation of the products

Experiment	RNA	Total incorporation (c.p.m.)	Anti-Lb serum precipitable radioactivity (c.p.m.)	% of total radioactivity precipitated by anti-Lb serum
1	Total polysomal RNA	10,716	2,679	25
2	*RNA not containing poly (A)	237	—	—
	*Total poly (A)-containing RNA	40,320	11,290	28
3	†9S RNA	27,250	15,465	57
	†12S RNA	38,000	12,780	33

RNA fractions were translated in the standard amino acid incorporating system from wheat embryos in a total volume of 200 μ l (experiment 2) or 400 μ l (experiments 1 and 3) as described in Fig. 1. After 60 min of incubation at 30° C an aliquot of the postribosomal supernatant was incubated with anti-Lb serum and processed as in Fig. 1. Aliquots treated with normal rabbit serum precipitated 150–250 c.p.m. in all cases. *Prepared by oligo(dT)-cellulose chromatography (Fig. 2).

†From sucrose gradients (Fig. 4).

carrier Lb on sodium dodecyl sulphate (SDS) gels (Fig. 1) we concluded that these polysomes contained mRNA which codes for soybean Lb.

RNA was extracted from nodule polysomes and fractionated on a column of oligo (dT)-cellulose¹². The RNA which eluted from the column with low salt buffer was found, by hybridisation with ³H-poly(U), to have most of the poly(A)-containing RNA (Fig. 2).

The RNA fractions from the column were tested for ability to support protein synthesis in the wheat embryo cell-free system, which in addition to ribosomes contains all the factors necessary for protein synthesis but has very little endogenous mRNA activity^{9–11}. Only the poly(A)-containing RNA supported the incorporation of ³H-leucine into released polypeptide chains (Table 1). Other RNA fractions were ineffective by themselves and were not

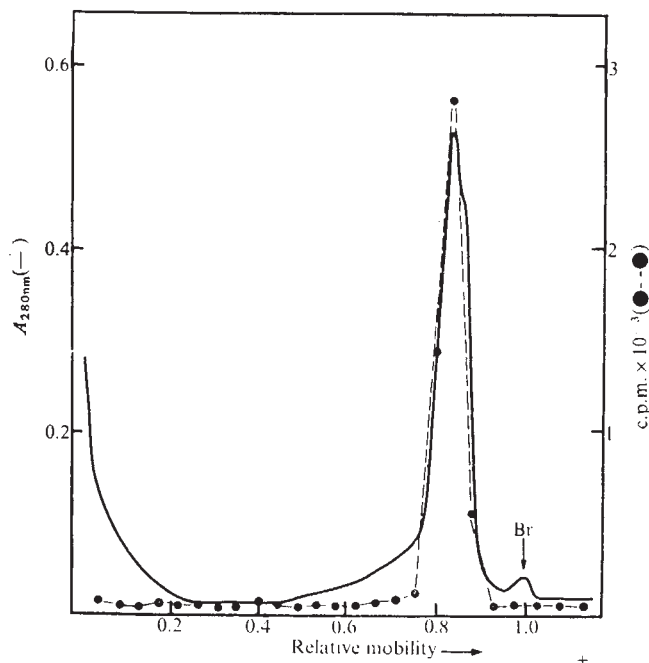


Fig. 3 SDS gel electrophoresis of the immunoprecipitated product from *in vitro* translation of leghaemoglobin mRNA. Poly(A)-containing RNA prepared from polysomes as in Fig. 2 was translated in the standard wheat embryo amino acid incorporating system in a total volume of 400 μ l (Fig. 1) containing 4 A_{260} units of wheat embryo ribosomes. After incubation, the postribosomal supernatant was reacted with anti-Lb serum and analysed in SDS gel as described in Fig. 1.

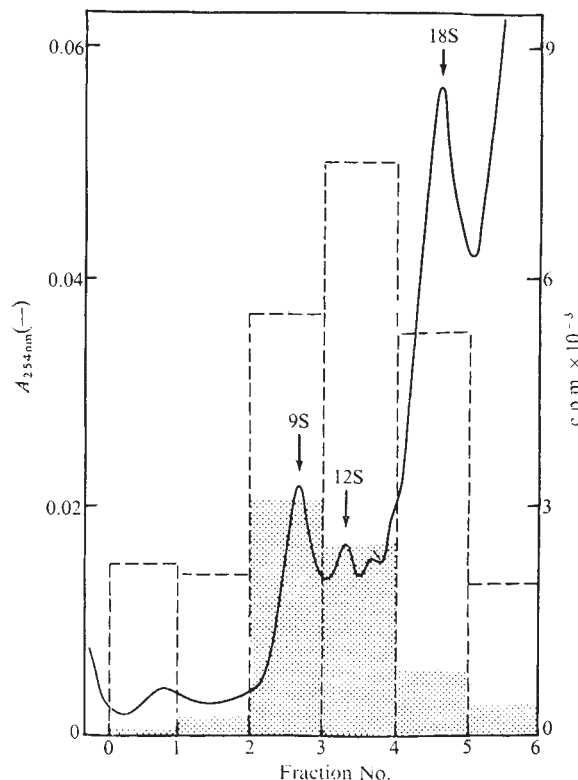


Fig. 4 Sucrose gradient centrifugation of poly(A)-containing RNA from soybean root nodules. Poly(A)-containing RNA was isolated (Fig. 2), mixed with 100 μ g of carrier RNA and layered on a 10 ml, 10–30% (w/v) sucrose gradient in 50mM Tris-acetate; 100 mM NaCl; 100 mM Na acetate, pH 8.5. Centrifugation was for 14 h at 39,000 r.p.m. at 2° C in a Beckman SW 41 rotor. Gradients were fractionated and A_{254} was monitored. RNA from each fraction was precipitated at –20° C for 24 h with 2.5 volumes of ethanol. The RNA was collected and dissolved in 40 μ l of water. Aliquots of these RNA fractions were translated in the standard amino acid incorporating system in a total volume of 200 μ l containing 2 A_{260} units of wheat ribosomes (Fig. 1). After incubation, an aliquot of each postribosomal supernatant was precipitated with hot 5% TCA and radioactivity was determined (total counts, open columns) while another aliquot was incubated with anti-Lb serum (shaded columns) for 60 min at 30° C. The immunoprecipitate was washed twice with phosphate-buffered saline and collected on Whatman GF/C filters. Filters were washed with 5% TCA, dried and their radioactivity determined (anti-Lb counts).

inhibitory when added together with poly(A)-containing RNA. Of the soluble radioactive products synthesised in the presence of the poly(A)-containing RNA, 25–30% was precipitated by anti-Lb serum. The immunoprecipitated radioactivity migrated with carrier Lb on SDS gels (Fig. 3), similar to the products made by total polysomes (Fig. 1).

Analysis of the poly(A)-containing RNA by sucrose gradient centrifugation showed that it had mainly 9S and 12S components (Fig. 4). Translation of the individual fractions from the gradient demonstrated that the 9S and 12S fractions were the most active in supporting protein synthesis in the wheat embryo system and the products from these fractions had the highest reactivity with anti-Lb serum (Fig. 4 and Table 1). The S value of these RNA fractions was confirmed by electrophoresis in 2.8% acrylamide gels¹³. The position of RNA in the gels was determined by ³H-poly(U) hybridisation to fractions eluted from 2 mm gel slices.

Since the two species of Lb in soybean root nodules (LbS and LbF) are equally reactive with Lb antisera and migrate together in SDS gels (Figs 1 and 3) it was not possible to determine whether the poly(A)-containing mRNA codes for one or both leghaemoglobins. To answer this

question the radioactive product was analysed with carrier LbS and LbF by disc electrophoresis¹⁴. Figure 5 shows that the bulk of the radioactivity accompanied the LbS whereas relatively little was associated with the LbF. The fact that the radioactivity was in Lb was confirmed by its reactivity with anti-Lb serum after elution of the corresponding sections from a duplicate gel. At present we have no explanation for the selective synthesis of LbS, since extracts of nodules of all ages contain more LbF than LbS (our unpublished data).

For its *in vitro* translation, Lb mRNA does not seem to require any specific factors which may arise in the nodule as a result of the symbiosis. Preliminary experiments suggest that the translation of Lb mRNA in the wheat embryo cell-free system is not stimulated by the addition of haem. Furthermore, it is unlikely that the system responded to endogenous haem, since the addition of human serum albumin, which can bind haem avidly¹⁵, was without effect. It has also been shown that the translation of rabbit globin mRNA in the wheat embryo system does not require haem¹⁶.

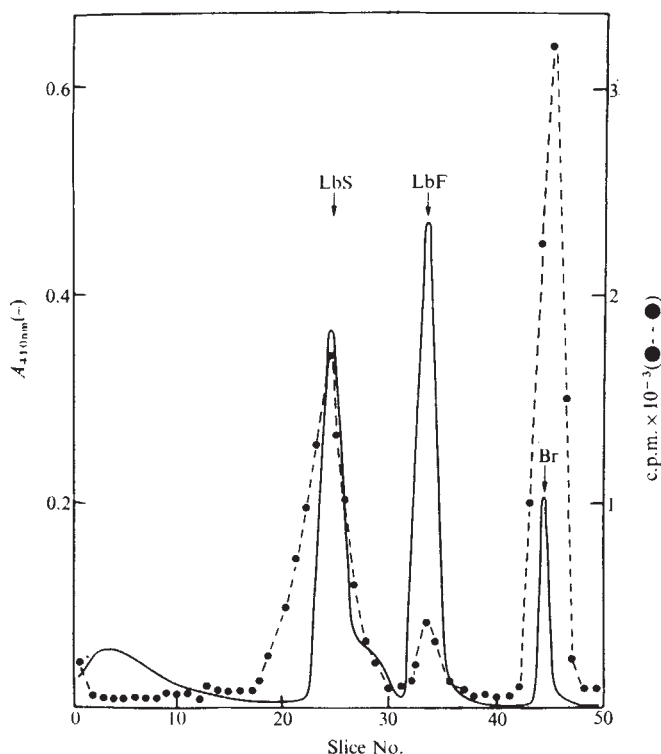


Fig. 5 Disc gel electrophoresis of the products made by poly(A)-containing RNA. The poly(A)-containing RNA from Fig. 2 was translated in the standard wheat embryo system as described in Fig. 1 and the postribosomal supernatant containing released products was mixed with 10 μ g each of LbS and LbF and analysed by electrophoresis (4 mA per tube) on 7.5% acrylamide gels¹⁴. After electrophoresis, gels were scanned at 410 nm in a Gilford spectrophotometer and their associated radioactivity was measured by fractionating the gels into 1 mm slices as described in Fig. 1. Br, bromophenol blue marker.

Although several eukaryotic mRNAs have been isolated and translated in various cell-free systems, this is the first report of the isolation and *in vitro* translation of a plant mRNA. Our experiments demonstrate that, like animal and viral mRNA, plant mRNA can be translated in a cell-free system derived from an unrelated species. Although isolated from a prokaryote-eukaryote symbiosis, Lb mRNA seems to be of plant origin since, like other eukaryotic messengers it contains poly(A) and is associated with 80S ribosomes¹⁷⁻²⁰. These results are consistent with other evidence that the

plant contains the genes for Lb^{4,5}. Hybridisation of Lb mRNA with DNA from uninfected soybean plants should provide definitive proof of the location of the Lb gene.

We thank Dr G. A. MacLachlan for laboratory facilities, Dr W. R. Jeffery for advice on the poly(U) hybridisation assay, and Dr D. Fromson for helpful discussions. This work was supported by grants from the National Research Council of Canada to H.M.S. and Dr G. A. MacLachlan.

DESH PAL S. VERMA

Department of Biology,
McGill University,

DUDLEY T. NASH

HERBERT M. SCHULMAN

Lady Davis Institute for Medical Research,
Jewish General Hospital and
Department of Biology, McGill University,
Montreal, Quebec, Canada

Received March 5; revised June 5, 1974.

- ¹ Bergersen, F. J., Turner, G. L., and Appleby, C. A., *Biochim. biophys. Acta*, **292**, 271-282 (1973).
- ² Virtanen, A. I., Jorma, J., Linkola, H., and Linnasalmi, A., *Acta chem. Scand.*, **1**, 90-111 (1947).
- ³ Cutting, J. A., and Schulman, H. M., *Biochim. biophys. Acta*, **192**, 486-493 (1969).
- ⁴ Dilworth, M. J., *Biochim. biophys. Acta*, **184**, 432-441 (1969).
- ⁵ Cutting, J. A., and Schulman, H. M., *Biochim. biophys. Acta*, **229**, 58-62 (1971).
- ⁶ Ellfolk, N., *Acta chem. Scand.*, **15**, 545-554 (1961).
- ⁷ Broughton, W. J., and Dilworth, M. J., *Biochim. biophys. Acta*, **317**, 266-276 (1973).
- ⁸ Palmiter, R. D., Oka, T., and Schimke, R. T., *J. biol. Chem.*, **246**, 724-737 (1971).
- ⁹ Seal, S. N., Bewley, J. D., and Marcus, A., *J. biol. Chem.*, **247**, 2592-2597 (1972).
- ¹⁰ Weeks, D. P., Verma, D. P. S., Seal, S. N., and Marcus, A., *Nature*, **236**, 167-168 (1972).
- ¹¹ Weeks, D. P., and Marcus, A., *Biochim. biophys. Acta*, **232**, 671-684 (1971).
- ¹² Aviv, H., and Leder, P., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 1408-1412 (1972).
- ¹³ Loening, U. E., *Biochem. J.*, **113**, 131-138 (1969).
- ¹⁴ Davis, B. J., *Ann. N. Y. Acad. Sci.*, **121**, 404-427 (1964).
- ¹⁵ Bunn, H. F., and Jandl, J. H., *J. biol. Chem.*, **243**, 465-475 (1968).
- ¹⁶ Efron, D., and Marcus, A., *FEBS Lett.*, **33**, 23-27 (1973).
- ¹⁷ Burr, H., and Lingrel, J. B., *Nature new Biol.*, **233**, 41-43 (1971).
- ¹⁸ Van de Walle, C., *FEBS Lett.*, **34**, 31-34 (1973).
- ¹⁹ Molloy, G. R., Sporn, M. B., Kelley, D. E., and Perry, R. P., *Biochemistry*, **11**, 3256-3260 (1972).
- ²⁰ Nakazato, H., Kopp, D. W., and Edmonds, M., *J. biol. Chem.*, **248**, 1472-1476 (1972).

Differential staining of human and mouse chromosomes in interspecific cell hybrids

VIRUS-MEDIATED fusion of somatic cells to produce interspecific cell hybrids has proved to be a most valuable technique for biological research¹. Such crosses often undergo an initial fairly random loss of many chromosomes of one parent before karyotypic stability is reached. This property can be used to generate clones of cells each containing a small selection of human chromosomes in a background genotype of some other species. Comparisons of such cell lines provide a form of segregation analysis which can substitute for more traditional genetic methods, particularly in studies on man where classical genetic methodology is difficult to apply.

Among the many interesting applications of this technique, a particularly prolific field has been the mapping of the human genome². By studying the cosegregation of human gene products in a number of hybrid clones, linkage of the gene loci determining those products can be inferred.

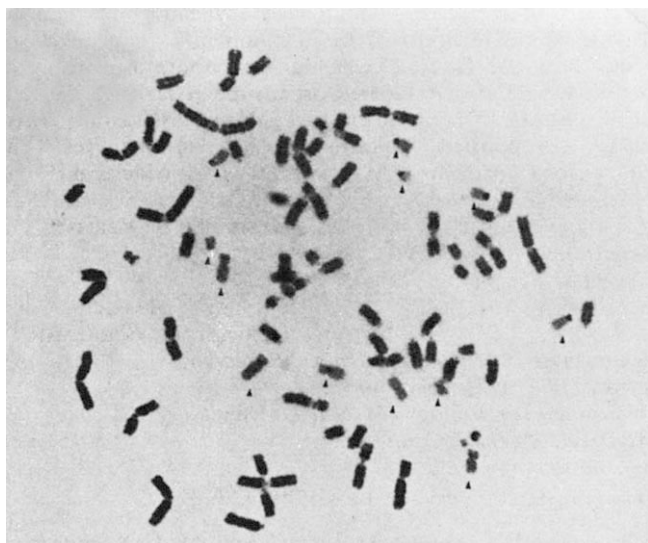


Fig. 1 Metaphase of interspecific hybrid between mouse cell line (IR) and human lymphocytes. Human chromosomes (arrowed) are pale blue, some having red paracentromeric heterochromatin. Mouse chromosomes have pale blue centromeric heterochromatin and red euchromatic arms.

If the karyotypes of the cell lines are established, gene loci can be assigned to particular chromosomes.

The availability of well characterised cell lines and extensive genetic knowledge of the species, make mouse cells particularly suitable as 'carriers' in such studies. A major disadvantage, however, is the difficulty of characterising the karyotypes of mouse-human cell hybrids. Human acrocentrics, and abnormal chromosomes, are difficult to pick out of the complex and variable mouse background. We have been investigating means of differentiating human from mouse chromosomes, to allow a fairly rapid screening of cell lines for the number and morphology of human chromosomes carried. With this knowledge, formal identification of the individual chromosomes concerned using various chromosome banding techniques is greatly simplified. We report here that mouse chromosomes, and the chromosomes of permanent mouse cell lines, show an almost exact reversal of the Giemsa-11 staining pattern found for human chromosomes, and that these staining characteristics are retained in mouse-human hybrid cells.

The Giemsa-11 staining technique^{3,4} highlights specific regions of constitutive heterochromatin in the human karyotype. In optimal preparations, the chromosomes are a

bluish colour, with red differentiation of paracentromeric regions of chromosomes 1, 4, 5, 7, 9, 10, 13, 14, 15, 20, 21 and 22. Parts of the Y chromosome are also stained. Mouse chromosomes stain a dark magenta, with pale blue centromeres. In hybrids, those human chromosomes which contain G-11 positive material are most readily identified, being pale blue with red paracentromeric regions (Fig. 1). Even those human chromosomes such as the X, which have no G-11 positive material, can be differentiated from the surrounding mouse chromosomes by their lighter colour, and by the absence of the markedly paler centromere regions characteristic of mouse chromosomes.

The staining procedure is similar to that used on diploid human cells. Colcemid-arrested metaphases are treated for 15–20 min in 0.075 M KCl, fixed in 3:1 methanol:acetic acid, and air dried. Flame dried slides can also be used. Slides are left for about 1 week at room temperature, or for 2 d in a dry oven at 60°C, before staining. The staining solution is a 1/50 aqueous dilution of Giemsa (Harleco), adjusted to pH 11.0 with NaOH. Optimal staining is usually achieved in 10–20 min. Slides are then rinsed in pH 6.8 phosphate buffer and allowed to dry. Unmounted slides can be examined and photographed with oil immersion objectives. After removing the oil with xylene, they can be destained in two changes of acetic methanol and satisfactorily restained with quinacrine or G-banded using trypsin⁵.

As with all chromosome banding techniques, the quality of staining is somewhat erratic. But altering the staining time, and the pH and dye concentration of the staining solution, we have achieved success in 14 out of 15 hybrid clones studied. The identity of the chromosomes concerned

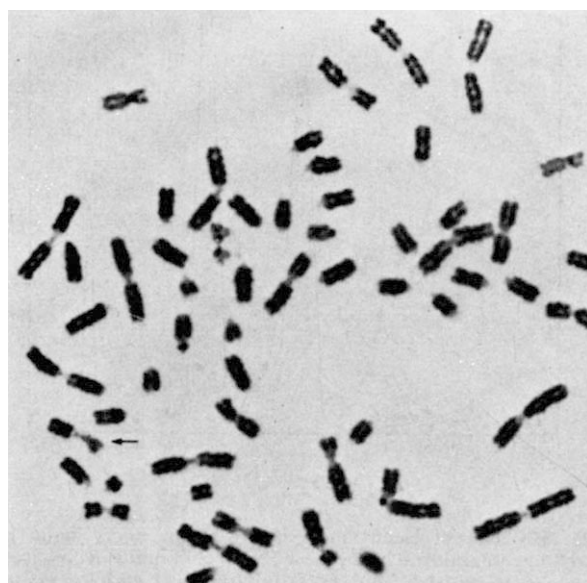


Fig. 3 Partial metaphase of mouse-human hybrid (clone 2W1-R70) showing possible interspecific translocation chromosome (arrowed).

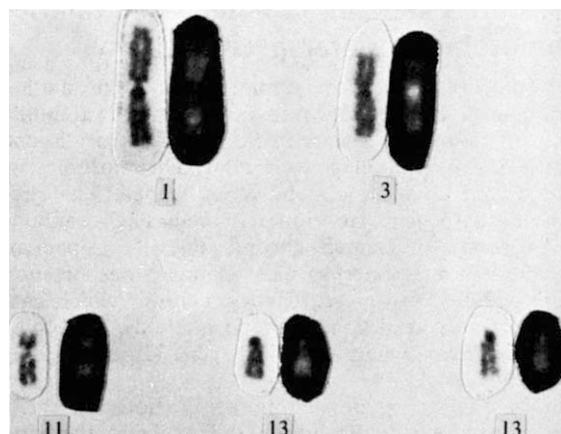


Fig. 2 Human chromosomes from a single mouse-human hybrid metaphase, identified by G-11 staining (left) and restained with quinacrine (right).

has been established by re-examining previously photographed metaphases under quinacrine fluorescence, (Fig. 2), as well as by formally karyotyping Giemsa banded cells of the same clones. One clone, known on biochemical evidence to contain only part of a human X chromosome, was found to have a chromosome which stained in part as a mouse chromosome and in part as a human chromosome (Fig. 3). The pale staining of human euchromatin cannot be distinguished from mouse constitutive heterochromatin and although this chromosome has not been seen in either the parent mouse line or any of the 11 other hybrid clones derived from it, its characterisation as a mouse-human translocation cannot yet be regarded as final.

The physicochemical basis of the G-11 staining reaction remains obscure. On human chromosomes, it was previously noted⁷ that staining in alkaline Giemsa rapidly leads to an overall pale blue coloration suggestive of methylene blue, with the subsequent development of specific red staining at particular chromosomal sites. This accords with the work of Sumner and Evans⁸ on G-banding, which led them to propose that the magenta colour of chromosome bands stained with Giemsa was due to the *in situ* formation of a molecular complex of methylene blue and eosin. Giemsa-banding can also be produced by simple manipulation of the pH of the staining solution⁷, suggesting that as the alkalinity of the stain is raised, an increasing amount of human chromatin loses the capacity to form such magenta dye complexes.

The ability to form red dye complexes at high pH is clearly not a particular property of constitutive heterochromatin or of highly repetitive DNA sequences, since mouse heterochromatin exhibits the same colour changes as human euchromatin even when the chromosomes are in the same cell. It seems likely that the change in staining is not due to a direct influence of pH on the process of dye binding, but is mediated by an alteration in the structure of particular regions of chromatin. This could be as simple as a disruption of the compact coiling of the chromatin fibrils, due to an effect of pH on associated structural proteins, but the real nature of the process is still completely unknown.

It is, however, clear that the G-11 technique can serve as a most useful method for the primary analysis of the chromosomes of human-mouse cell hybrids. Coupled with re-examination of photographed metaphases under quinacrine fluorescence, it has provided us with an acceptable technique for the identification of human chromosomes against a mouse chromosome background. Whether it will prove useful for other combinations of species has not yet been determined.

This work was supported by a programme grant from the Medical Research Council.

MARTIN BOBROW
JANE CROSS

Genetics Laboratory,
Department of Biochemistry,
South Parks Road,
Oxford, UK

Received June 18; revised July 5, 1974.

¹ Harris, H., *Cell Fusion* (Harvard University Press, Cambridge, 1970).

² *New Haven Conference: First International Workshop on Human Gene Mapping. Birth Defects: Original Article Series X:3.* (The National Foundation, New York, 1974).

³ Bobrow, M., Madan, K., and Pearson, P. L., *Nature new Biol.*, **238**, 122-124 (1972).

⁴ Gagne, A., and Laberge, C., *Expl Cell Res.*, **73**, 239-242 (1972).

⁵ Seabright, M., *Lancet*, **ii**, 971-972 (1971).

⁶ Sumner, A. T., and Evans, H. J., *Expl Cell Res.*, **81**, 223-236 (1973).

⁷ Patil, S. R., Merrick, S., and Lubs, H. A., *Science*, **172**, 821-822 (1971).

lymphoma (BL), but the potential of MD as a model of human lymphoproliferative disease and of herpesvirus-induced neoplasia has not been fully realised. One obstacle has been the lack of any established cell lines¹. This contrasts with studies on BL, where, in the absence of suitable experimental animals, attention has been focused on the growth of tumour cells *in vitro*². A continuous cell culture from a lymphoma of MD has recently, however, been reported³. We now report the establishment of two cell lines from MD lymphomas and their characterisation as T cells.

The approach used was that found to be successful in the long term culture of lymphocytes from BL biopsies⁴. Ovarian lymphomas were collected from experimentally infected birds at about 5 weeks of age. Single cell suspensions were prepared either by gentle teasing of the tumours or by agitation in 1% trypsin. The suspensions were adjusted to 5×10^6 cells ml⁻¹ and cultures of 5 ml volume were incubated in screw cap glass universal bottles at either 37° C or at 40° C. Cultures of lymphocytes separated from heparinised blood samples

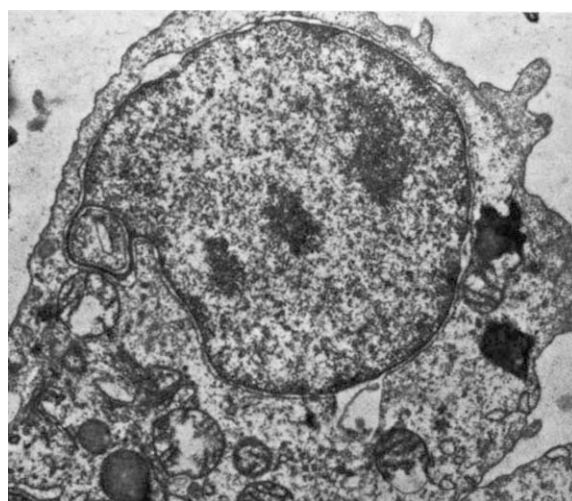


Fig. 1 Typical lymphoblast of HPRS Line 1. The rounded nucleus has a projection of the nuclear envelope. The cytoplasm contains many ribosomes, several mitochondria and osmiophilic bodies, but sparse endoplasmic reticulum. ($\times 11,250$)

were initiated in a similar way. The medium used was RPMI 1640 supplemented with 20% foetal calf serum (FCS), and 10% tryptose phosphate broth. All cultures were set up in replicates according to the number of cells available.

Cultures were refed by replacing half of the supernatant medium, taking care not to disturb the cells, with fresh medium every 2 d for the first week of culture, and thereafter weekly.

Several cultures gave rise to a profuse growth of adherent fibroblast-like cells. Cultures were examined regularly for evidence of the growth of free floating cells. One culture derived from an ovarian tumour was found to be growing in this manner 31 d after initiation. This culture has now been growing for 29 weeks and is designated 'HPRS Line 1'. A second culture (HPRS Line 2), also from a lymphoma, was found to be multiplying 92 d after initiation. This culture has now been growing for 13 weeks. Cultures were divided into two every 7 or 10 d according to their appearance.

In the growth of both cultures a high proportion of dead cells has been consistently observed. Medium supplemented with 30% FCS or with 15% FCS and 15% chick serum supported growth, but did not correct this problem. The cells were easily damaged by handling. Growth was better at 40° C than at 37° C.

The cells were never attached to glass. They appeared singly

T lymphoblastoid cell lines from Marek's disease lymphomas

MAREK'S disease (MD) is a contagious lymphoproliferative condition of chickens caused by a herpesvirus. It has the distinction of being the first commonly occurring tumour to be controlled by vaccination. In its complex pathology and aetiology the disease bears remarkable similarities to Burkitt's

or in pairs but not in clumps. Most cells were round but a few had projections making them elongated. The cells ranged in size, when measured in the living state, from 5 μ m to 12 μ m with a mean of 8 μ m. In smears the cells had large nuclei and a rim of intensely basophilic cytoplasm containing vacuoles. Some large cells contained two nuclei. Nucleoli were not seen.

Ultrastructurally HPRS Line 1 cells were similar to cells seen in MD lymphomas (Fig. 1). The nucleus was round or oval and sometimes slightly indented. A nucleolus was occasionally seen and about 3% of the cells showed projections of the nuclear envelope (Fig. 1). The cytoplasm contained many ribosomes, some of which were in the form of polyribosomes. Several mitochondria were present, but the endoplasmic reticulum, which was often rough surfaced, was sparse. A Golgi apparatus was seen in some cells. Centrioles were fairly frequent and were often accompanied by spindle tubules. Small, often irregularly shaped osmiophilic bodies, probably of lipid were normally present in the cytoplasm. Occasional vacuoles were seen, and a small number of cells

showing smooth membrane staining with capping, typical of T cells. Line 2 cells have been examined for membrane fluorescence on one occasion: 100% stained for T cell determinants, and 0% for B-cell determinants. The identification of the cell lines as T cells is supported by the ultrastructural studies which showed the presence of abundant ribosomes and sparse endoplasmic reticulum.

The majority of cells in Marek's disease lymphomas are of thymus origin^{5,6}. The role of these cells in the histopathogenesis of the disease is not clear. The lymphoid system may be involved in the production of tumours by the malignant transformation of lymphoid target cells (intrinsic mechanism), or by the participation of lymphocytes in an excessive proliferative response to new viral antigen (extrinsic mechanism)⁷. Since MD usually has a multifocal origin, an extrinsic mechanism is obviously a possibility. There is, however, a poor correlation between the presence of viral antigen and the site of tumour development⁸. Moreover, BL is also frequently multifocal and yet is a uniclonal disease; this implies that the successful development of a neoplastic clone is a relatively rare event,

Table 1 Percentage of cells showing specific membrane fluorescence*

Antiserum	HPRS Line 1	Thymus suspension	Bursa suspension	Blood lymphocyte	MD lymphoma	MD virus plaques in CKC
Anti-thymus	99.5 $\pm$ 0.3	93.9 $\pm$ 2.3	4.0 $\pm$ 0.4	73.5 $\pm$ 2.8	76.6 $\pm$ 3.9	—
Anti-bursa	1.1 $\pm$ 0.4	4.3 $\pm$ 0.3	94.0 $\pm$ 0.9	21.8 $\pm$ 3.3	20.4 $\pm$ 2.4	—
Anti-tumour	96.3 $\pm$ 1.2	0	0	1.3 $\pm$ 0.2	35.1 $\pm$ 9.6	—
Anti-MDV	1.0 $\pm$ 1.0	0	0	0	2.8 $\pm$ 1.8	+++

* Mean of five determinations. $\pm$ Standard error.

had parallel arrays of annulate lamellae in the cytoplasm. Cells containing immature herpesvirus particles were seen rarely.

HPRS Line 1 cells acted as plaque-forming infectious units to chick kidney cell monolayers. Estimates of infectivity, each on several different subcultures, were made at four different times. These gave values of 80, 1,048, 233, 1,278 plaque-forming units per million viable cells. The cell lines do not produce cell free virus.

Two cultures capable of long term growth have arisen from a total of 120 cultures, derived from 49 tumours and 11 blood samples. A further 32 lymphocyte preparations from normal blood failed to grow in long term culture. This contrasts with the relative ease with which lymphoblastoid lines may be established from human blood and BL biopsies, and suggests that the method described is not optimal, though it may be a reflection of the notorious difficulty of establishing avian cell lines¹.

Suspensions of living HPRS Line 1 cells were examined for membrane antigens using the indirect fluorescent antibody technique⁵. The specific antisera were raised in chickens against HPRS 16 infected chick kidney cells (anti-MDV), and in rabbits against suspensions of MD lymphoma cells (anti-tumour), normal thymus cells (anti-T) and normal bursa cells (anti-B). All antisera had been extensively cross absorbed. The results are shown in Table 1. The anti-B and anti-T sera were specific as assessed by the staining of viable thymus or bursa lymphocytes. These sera stained B and T cells respectively in the peripheral blood. The anti-MDV serum was specific for virus plaques in chick kidney cell cultures. The high proportion of lymphoma cells and HPRS Line 1 cells staining with the anti-tumour antiserum suggests the presence of a tumour-specific antigen, though the staining of a small number of normal lymphocytes makes the specificity of the reaction questionable. The results indicate that such a tumour-specific antigen is not identical to the known virus-associated antigens. Line 1 cells stained for T-surface determinants,

and may depend upon an infrequent accident of interaction between virus and cells of an appropriate transformation type¹. Tumour-specific antigens have been reported⁹ in MD: the results of our fluorescent antibody studies suggest that the cell lines carry membrane tumour-specific antigens. The presence of MD virus in the cells parallels the role of EB virus in the proliferation of human lymphocytes *in vitro*. We suggest that malignant transformation of thymus-dependent lymphocytes is necessary for the development of lymphomas in Marek's disease.

We thank Miss B. Parish and A. Kidd for technical assistance.

P. C. POWELL
L. N. PAYNE
J. A. FRAZIER
M. RENNIE

Houghton Poultry Research Station,
Houghton, Huntingdon, PE17 2DA, UK

Received May 23, 1974.

- Klein, G., in *Oncogenesis and Herpesviruses* (edit. by Biggs, P. M., de-Thé, G., and Payne, L. N.), 501-515 (International Agency for Research on Cancer, Lyon, 1972).
- Epstein, M. A., in *Burkitt's Lymphoma* (edit. by Burkitt, D. P., and Wright, D. H.), 148-157 (Livingstone, Edinburgh, 1970).
- Akiyama, Y., Kato, S., and Iwa, N., *Biken's J.*, **16**, 177-179 (1973).
- Epstein, M. A., and Barr, Y. M., *Lancet*, **i**, 252-253 (1964).
- Hudson, L., and Payne, L. N., *Nature new Biol.*, **241**, 52-53 (1973).
- Rouse, B. T., Wells, R. J. A., and Warner, N. L., *J. Immun.*, **110**, 534-539 (1973).
- Payne, L. N., in *Oncogenesis and Herpesviruses* (edit. by Biggs, P. M., de-Thé, G., and Payne, L. N.), 21-37 (International Agency for Research on Cancer, Lyon, 1972).
- Payne, L. N., and Rennie, M., *J. natn. Cancer Inst.*, **51**, 1559-1573 (1973).
- Ishikawa, T., Naito, M., Osafune, S., and Kato, S., *Biken's J.*, **15**, 215-222 (1972).

reviews

Jacob as historian and biologist

The Logic of Living Systems. By Francois Jacob. Translated by Betty E. Spillmann. Pp. viii+348. (Allen Lane: London, 1974.) £4.

THIS book is a history of knowledge and ideas on reproduction and heredity. It is intended by its author to be not merely a narrative account but a history which is in some sense explanatory, a history which shows how this knowledge was generated and how it changed.

Jacob outlines his historiographic principles in the introductory "programme" where he rejects "reverse history"—which simply studies the succession of ideas resulting in our present views—as inadequate. Rather, Jacob argues, a history of scientific knowledge and ideas must seek "to discover how objects become accessible to investigation"; an "analysis of the nature of the object is required and of the attitudes of the investigators, their methods of observation and the obstacles raised by their cultural background". Using this approach, Jacob maintains, one no longer observes a linear sequence of ideas—"the confident march of progress"—each derived from its predecessors by a sort of evolutionary transformation, but instead a series of more or less self-contained and discontinuous "domains which thought tries to explore, where it seeks to establish order and attempts to construct a world of abstract relationships in harmony not only with observation and techniques but also with current practices, values and interpretations". For an object to be accessible to investigation a theory able to accommodate it must exist and "each period is characterised by a range of possibilities defined not only by the current theories and beliefs but also by the very nature of the object". This range of possibilities is the "domain", the territory within which reason can operate, and it is from these possibilities that theoretical "solutions" to problems are chosen. When new theories are accepted they reorganise "the domain of the possible", modify ways of looking at things and bring to light new relationships and objects. Historiographic principles of this sort, Jacob maintains, will result in a history which is concerned with "specifying the various stages of knowledge, defining the trans-

formations and revealing the conditions which enable objects and interpretations to enter the field of the possible".

Jacob clearly has given much thought to the problems of writing history and the conceptual outline in his programme will be familiar to those who have some knowledge of that heterogeneous body of thought loosely labelled 'structuralism'. His specific historiographic principles immediately bring to mind the work of Michel Foucault (and, more remotely, T. S. Kuhn) and it is not difficult to see the influence of Foucault's ideas on this book, even though Jacob makes no reference to him, or indeed to any contemporary historian.

Jacob's careful statement of his historiographic principles and their nature led me to expect a work that would be comparable to the work of Foucault and to that of those other French students of cultural phenomena who are working within a broadly similar framework. I expected a history of biology radically different from those produced within a more conventional, empirically oriented, historiographic framework, one full of new and provocative insights and interpretations. I confess that I was disappointed. This is a book of some distinction, certainly; it contains much of great interest, it is thought provoking, it is written in a style which is lucid and elegant and it deserves to be widely read. Yet, it seems to me to fall considerably short of its stated goal; for Jacob has, in fact, produced a book which is almost a reverse history, and one that is relatively conventional, even old fashioned in some respects, especially as regards its rigorous internalism.

It is not easy in a short review to do more than suggest why I found the book disappointing. It is a complex work which resists easy generalisations and in what follows I am conscious that I have not done justice to it and that it requires and deserves a more detailed analysis.

The book's shortcomings arise, I suspect, because of a tension or conflict between Jacob the biologist and Jacob the historian. The first Jacob has made significant contributions to present knowledge and obviously has a certain commitment to it; he is concerned with the diachronic and with progress. The second Jacob, in his role

as historian, is concerned to do justice to his predecessors; he is interested in the synchronic but wary, perhaps, of the danger of relativism. I suspect that the first Jacob had the upper hand in the writing of this book.

In his discussion of the development of evolution theories, he remarks that "the role that is attributed to the past... is necessarily conditioned by the way in which the present is viewed and interpreted". This remark could be applied to his own book, for in the last chapter he presents an interpretation of living systems as a hierarchical arrangement of organisations, one "enclosed" within the other like a nest of "Russian dolls"; this structure provides the basis for Jacob's history which consists of a series of "stages of knowledge" in which each Russian doll is discovered. Beginning in the seventeenth century with a domain of thought which is concerned with "visible structure"—the arrangements of matter, he passes to a second domain of "organisation" which occurs at the end of the eighteenth century and is concerned with the invisible unity of the organism, a unity which is later dissolved into the properties and interactions of cells; at the beginning of the twentieth century is the third domain in which chromosomes and genes are possibilities and, finally, our domain, that of the nucleic acid molecule. Interposed between the discussions of the second and third stages is a chapter on "Time" in which ideas on the genesis and evolution of living things are considered.

The series of historical stages which Jacob has constructed on the basis of his picture of the structure of living systems is not simply a sequence; it is a progress towards something hidden and more fundamental; it represents a journey to the metaphorical centre of the organism; a centre which for Jacob is also metaphysical, the source of order and change: the genetic programme.

This basic conception of progress often seems to undermine Jacob's theoretical principles. He has a tendency on occasion to write with hindsight and to consider and pass judgement on past knowledge as simply a precursor of future, and therefore more fundamental, knowledge, rather than as a system possessing its own coherence. Consequently, there are places where past thought is very

simplified or even distorted. This progressive picture also results in the omission of thought which does not fit into the pattern, the most notable example being twentieth century developmental biology.

Jacob's rigorously internalist approach also seems to be in conflict with his historiographic principles and results in some disappointingly conventional interpretations. Evolutionary thought, for example, is described in the programme as a product of "mid-nineteenth century thought as a whole", yet it is actually discussed in a very conventional fashion as an internal problem in biology, centring on the issue of the contingency of the living world, with a reference to similar ideas in contemporary statistical mechanics. This, in my view, is neither an adequate nor a particularly illuminating account of a transformation in thought involving ideas on God, man, nature and society and their inter-relations.

On the other hand, problems also arise when Jacob applies his historiographic principles. The notion of relatively discontinuous domains of thought results in an historical picture in which the real continuities which exist are often obscured, and one which is often too schematic. His chapter on the "Visible Structure", which is concerned with seventeenth century thought, suffers as a consequence of this, even though parts of it are fascinating. His belief that "mechanism, despite its limitations, represented the only attitude compatible with knowledge of that time" and that other metaphysical principles were not scientifically productive but were merely of "ethical" or "philosophical" significance, seems to me to do violence to the richness and complexity of seventeenth century thought and results in misleading discussions of, for example, the discovery of microorganisms and the circulation of the blood. In these examples it seems that, although Jacob recognises that "objects" can form the subject of discourse in more than one domain, he believes that they are only accessible to investigation which is scientific and productive within a unique domain which is in "harmony" with their "nature". This presupposes that the "nature" of an "object" is completely independent of the theoretical framework in which that object is considered; a debatable assumption.

The final chapter, "The Integron", is clearly written by Jacob the biologist, and contains a discussion of some current views of the organisation of living systems. It is surprisingly uncritical and tends to confirm my suspicion that Jacob the biologist was the dominant personality in the writing of the whole book, for reverse or Whig

history is, by and large, intended to justify the present state of affairs and to encourage us to accept it uncritically. On the other hand, the sort of history in which Jacob the historian is interested, the sort which asks the question "How is it possible for anyone to believe that?", has the goal of making us ask the same question with respect to our own beliefs, and therefore, of freeing us from the spectacles of habit and from the tyranny of our own artefacts. Jacob seems to be aware of this but evades coming to grips with it. In the final paragraph of the book he writes: "There are perhaps other possible coherences in descriptions. But science is enclosed in its own explanatory system and cannot escape from it". This is to raise the possibility of freedom, and therefore the necessity of choice, and then immediately to reject it by picturing science as an autonomous and alien thing; the "Russian doll" as a Petrushka, an artefact with a life of its own. It is to evade the fact that we are responsible for all our artefacts, including our "beliefs" and our "knowledge".

GERALD WEBSTER

Stimulating immunity

Chemical and Biological Basis of Adjuvants. By P. Jollés and A. Paraf. Pp. viii+153. (Molecular Biology, Biochemistry and Biophysics, 13.) (Springer-Verlag: Berlin and New York, 1973.) £7.90.

At best the subject of immunological adjuvants consisted of a potpourri of related phenomena: the publication of this monograph has to some extent improved the situation.

The authors have striven hard to obtain order and consistency which they achieved with respect to the chemical basis of adjuvants, although it has been done by introducing a noticeable degree of repetition. I found the earlier chapters especially informative with regard to the adjuvant materials of mycobacterial origin. In the area of cellular immunology, Jollés and Paraf have not achieved such clarity and there is no coherent discussion of immunopotentiality by substances acting on populations of differentiating cells.

I found the last chapter on the practical uses of adjuvants very incomplete and nothing like as scholarly as the earlier chapters. It would have been useful to have had a more critical and detailed discussion of recent French work where certain bacterial vaccines (*C. parvum*; BCG) have been used in the treatment of leukaemias. Direct toxicity is an important consideration and is adequately discussed;

however only brief mention is made of the danger of adjuvants stimulating unwanted responses to auto-antigens.

The book is well produced, and I noted few typographical errors. Its usefulness might have been increased if the bibliography had been made to serve additionally as an author index, simply by adding the page numbers of each citation after each listed reference. 'Adjuvant workers' should find this monograph worth buying.

D. W. DRESSER

Natives of Canada

Athapaskan Adaptations. By James W. Vanstone. Pp. x+145. (Worlds of Man: Cultural Ecology Series.) (Aldine: Chicago, April 1974.) \$7.50 cloth; \$2.50 paper.

THE Athapaskan speaking peoples occupy a vast forest region of north-west Canada and most of the land surface of Alaska. As Walter Goldschmidt the series editor suggests in the foreword, Athapaskan culture was less dramatic than the Northwest Coast or the Plains types, but no less adaptive; "a cultural flexibility, a quiet competence, a sustained ability to blend in with local environment" (page vii). The Athapaskan groups are seen as small communities of hunters and fishermen adapted to salmon fishing in the Pacific drainage lowlands, collective caribou hunts in the open northern regions, and more solitary moose hunting in the dense forested southern regions of Great Slave Lake.

The book is an attempt to present a very generalised account of the ecology and social organisation of the Athapaskan groups at the time of contact with European and Russian entrepreneurs about 1750 AD. It is based on the current research of many scholars in the field, as well as those of the 1950s and the 1920s. In addition there are two principal sources from the early period, namely Samuel Hearne's account of his trip to the Coppermine River, NWT Canada of 1769-72 and Henry Allen's to the Ahtna of the Copper River, Alaska in 1885.

The linguistic units were divided into small, mobile task forces of hunters and fishermen, often widely separated if not isolated. Economic variations from this pattern were minor. But the author demonstrates an unaccountable variation of social practice. Among the 'Northern Athapaskan' some units have hereditary leadership, others have none. There is bilateral descent in some while others have matrilineal sibs; some have group and some have individual ceremonies. There is both matrilineal and flexible residence patterns, and a modified male vision quest is present in some.

Abandonment of the aged is present in most, but one region recognises special high status for the aged. Even the potlatch is present in some regions. Perhaps most significant is the range of marriage regulations, all the way from parallel cousin through cross-cousin to no-cousin marriages.

The picture of the Athapaskan before outside contact is obscured by the fundamental changes wrought by two hundred years of the fur trade, and one hundred years of Christian mission activities. Since the end of the Second World War, education, health facilities and police administration have resulted in the development of larger population centres, the construction of many buildings, and the presence of alien supervisors in both Canada and Alaska.

The author describes in detail the settlement of Old Crow in the Yukon which exemplifies this recent change. He suggests "the community members almost never join in any work for the community as a whole because there are no leaders who can organise activity for the general welfare and improvement of the settlements" (page 117). Thus the members of Old Crow "have not been successful in developing social mechanisms to cope with the problem of living in large, permanent settlements" (*ibid.*). Although this is a general phenomenon throughout northern Canada, the author indicates the cause of failure is that "traditional social organisation emphasised the freedom of the individual family and the authority of the family head" (page 118).

Surely to seek causes in traditional culture forms is an oversimplification at best. Rather, the large permanent settlements based on imported funding and direct administration by aliens have emasculated the indigenous people. Most of Canada's indigenous peoples are in a similar position to these at Old Crow and are equally politically inarticulate. Many observers feel that the only change possible will be through the new regional organisations of Indians, Metis and Inuit (Eskimos). Dr Vanstone's views on their organised protests and negotiations with governments would have been valuable.

R. W. DUNNING

Structure of the cerebellum

Cerebellar Cortex: Cytology and Organisation. By Sanford L. Palay and Victoria Chan-Palay. Pp. xii+348. (Springer-Verlag: Berlin and New York, 1974.) DM156; \$64.

For the neuroanatomists of the nineteenth century, the cerebellum was a source of fascination, providing an unending series of vexing problems and forming the battle ground between the

neuronists, who believed that each nerve cell was a discrete entity, and the reticularists who argued that all the nerve cells in the brain were fused together into one extensive network. The reasons for the fascination and the conflict become quickly apparent to any reader of this superb book; six types of neurone, including the largest and the smallest types in the central nervous system, regularly interconnect to process the inputs conveyed by the two main afferent fibre systems. Furthermore, the modes of terminal arborisation of the fibres in the cerebellum exemplify both the simplest and the most complex types found in the nervous system.

During the latter part of the last century, the various cell types of the cerebellum were quickly recognised and, with the new staining technique, their processes were gradually characterised. All the great neuroanatomists of the past contributed to this achievement but it was Ramón y Cajal who in 1888 gave precise descriptions of all six cell types, as well as the 'climbing' and 'mossy' fibres, which form the two input pathways. But there remained a host of important questions which could not begin to be answered until the electron microscope had been developed.

This book takes up the problems Ramón y Cajal left unanswered. Starting from the Purkinje cell, the authors describe in successive chapters all the remaining cell types and afferent fibres. Each chapter begins with a brief historical account of the discovery and ensuing controversies, together with an assessment of the original descriptions. Then the up-to-date knowledge is lucidly presented. The book may be read at various levels. For the uninitiated camera lucida drawings of different neurone types and electron micrographs accompanied by detailed expositions of ultrastructural features will provide a glimpse of the basic 'wiring diagram', and of its extraordinary intricacy. For neuroscientists, it will be an invaluable source book. It provides a comprehensive overview of the important facts unravelled in the past two decades. These include evidence derived from both the optical and electron microscopes, as well as the electrophysiological techniques. In addition, numerous details of previously unpublished observations of the authors are incorporated.

This book is a credit to its senior author, whose sustained interest and imaginative application of current techniques have brought about a significant advancement in our understanding of the structure of the cerebellum. It is a worthy successor to the classic works of the Ramón y Cajal era.

SHIN-HO CHUNG

Uncontrolled eddies

The Analysis of Eddy Currents. By Richard L. Stoll. Pp. xii+128. (Monographs in Electrical and Electrical Engineering.) (Clarendon: Oxford; Oxford University: London, January 1974.) £3.50.

DESPITE the crucial importance of eddy current phenomena, in devices like the induction motor, the undergraduate introduction to the subject is necessarily very limited. It depends largely on equivalent circuit concepts, and assumes that the eddy currents are confined to insulated conductors, and are under the control of the designer. Recent developments in large, electrical machines and electromagnetic devices have added to the range of problems in which the eddy current flow paths are not so clearly defined or controlled, and have provided a fresh impetus to their study. In general, the problems demand a direct solution of the underlying field equations, yet even the simple methods and solutions receive scant attention in most textbooks on electromagnetic fields, and descriptions of more recent work are confined to the original references. Dr Stoll's book fills an important gap. It combines in a condensed and convenient form a description of basic analytical methods, and an introduction to numerical techniques by which the more complex problems, including flux and current penetration into iron, can be solved by computer. It is an essential addition to the bookshelf of those faced with the need to analyse eddy current problems, particularly at low (that is power) frequencies.

Dr Stoll writes as a specialist who has made considerable contributions to his subject, and he has included a great deal of useful material in 125 pages. Yet his treatment is admirably lucid, and the relevant parts of the book are well suited to the needs of electrical engineering and physics undergraduates and others seeking an introduction. The description of numerical methods is necessarily selective, in an area which is rapidly developing, and although it is a pity that topics such as finite-element and integral methods have had to be referred to only briefly, the material is well chosen for those whose principal interest is in applying the methods, rather than developing them. The book meets admirably its intended purpose, which is to provide an understanding of the more important methods and a bridge to specialist literature. It is well produced, and is a pleasure to read, although at some points a less condensed treatment, with additional diagrams, might be helpful to students with only a limited familiarity with electromagnetic field equations.

C. J. CARPENTER

Mechanisms in minds

Current Biochemical Approaches to Learning and Memory. Edited by Walter B. Essman and Shinshu Nakajima. Pp. ix+205. (Monographs in Modern Neurobiology.) (Spectrum: Flushing, New York; distributed by Halsted (Wiley), March 1974.) £8.40.

THIS book consists of an introductory chapter by the two editors, and eight chapters devoted to current research in the area of biochemistry and learning. The first chapter is a short, readable and sensible historical review, and the only chapter for which there would seem to be any excuse for publication. The remaining chapters are brief, superficial, badly written, and poorly edited; mistakes in spelling and punctuation, and peculiarities of grammar are common throughout, particularly in those chapters by authors whose first language is, as is all too evident, not English. There is not even any consistency in the way in which references are set out, some authors giving titles of papers, and others, page numbers only.

The brevity of the chapters has two unfortunate effects; first, no attempt is made to place the research described in a wider setting, by showing its relationship to the often very large body of relevant material from other laboratories. Bogoch, for example has a reference list of 29 entries, 28 of which are to his own work; Tsukada has one reference (to Tsukada). In some cases, there is a distinct impression that the authors are simply unaware of the research carried out in other laboratories—the chapter by Ilyutchenok and his colleagues on cholinergic mechanisms does not refer at all to Carlton, and only in passing to Deutsch.

The other consequence of brevity is that the evidence in support of authors' claims is very rarely presented in sufficient detail to allow its evaluation, and frequently no very clear idea emerges of exactly what procedures were used. What little hard evidence is provided is distinctly discouraging, displaying as it does, in almost every chapter, a cavalier attitude to statistics. For example, Fjerdingstad, in the only chapter on transfer of learning, applies one-tailed *U* tests to each of several test sessions on the same animals, without any regard to the fact that acceptable significance levels change drastically with such a technique. Whether such considerations would influence the editors is open to doubt. Nakajima in his chapter reports an experiment, with six groups of eight animals each; although he first states that the number of animals is insufficient for statistical analysis, he nevertheless goes on to tell us that the results "suggest" one conclusion and "also indicate" another.

In short, this is a book that should never have been published. It is extremely expensive, and one can only hope that few institutions, and fewer individuals, will feel obliged to purchase it.

EUAN M. MACPHAIL

Plants in action

Dynamic Aspects of Plant Ultrastructure. Edited by A. W. Robards. Pp. xii+546. (European Plant Biology Series.) (McGraw-Hill: London and New York, June 1974.) £8.95.

DR ROBARDS has brought together seventeen distinguished workers to write fifteen chapters in his symposium. It is difficult to fault either the credentials or the international flavour of the chosen authors. The advantage of the symposial approach is that each section can be written at the front of knowledge of that topic and, given a firm editor which has here been achieved, a relative evenness of treatment is possible. My quarrels with statements made by individual authors are of the level to be settled at small conferences and not of major substance. The disadvantage of the symposial approach is that each author is constrained to write within a precise framework and this inhibits cross correlations or interpretative sections on, for example, overall control mechanisms or communication between cells. A book of this type, excellent

as it is, must therefore be read in conjunction with single-author works at the same level.

Errors are few and good references numerous. Economies such as the lack of line 'justification' are justified and bother me not at all. But was it worth putting in some, only just relevant, expensive colour plates and printing all the rest of the plates on muddy matt paper even muddier than Dr Robards's previous text in this series? It does hold the price down to a reasonable level, but it must have enraged the authors of some of the beautiful micrographs included.

B. E. JUNIPER

Techniques for analysis

Analytical Chemistry: An Introduction. By Donald J. Pietrzyk and Clyde W. Frank. Pp. xx+667. (Academic: New York and London, April 1974.) \$13.95; £6.70.

THE course described in this book was originally designed as a one-semester introductory course in analytical chemistry for subsidiary students, but it is now intended for students taking chemistry as their major subject. There are four main areas: spectroscopy, acid-base methods, potentiometry with special reference to ion selective electrodes and chromatography. These topics are subdivided into further parts.

Analytical chemistry now covers such a broad area that only in specialised courses can it be treated in depth. An introductory course should set out to show what techniques are available and provide some guidance as to which are suitable for a particular purpose. Generally, in an introductory course the main limitation is time and therefore a very careful selection has to be made.

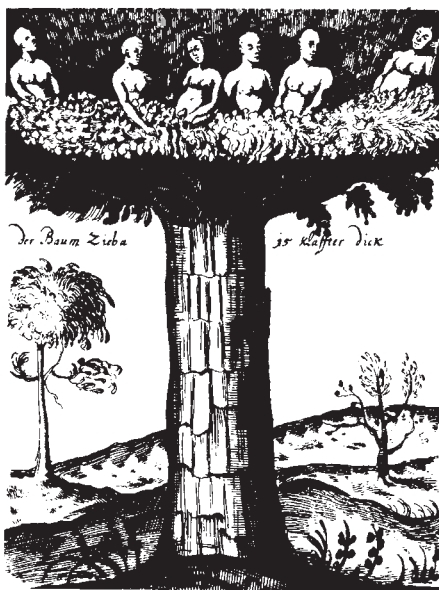
The authors of this book have obviously given a great deal of thought to the problem, for this is a well balanced course presenting information on all the basic methods and techniques of analytical chemistry. A balanced picture of the respective parts played by instrumental and classical analysis is also provided.

Some excellent exercises are described and possibly the only criticism to be made here is the omission of one on solvent extraction; it is a curious fact that most teaching texts on analytical chemistry avoid this exercise, despite the importance of the subject. Another criticism is that a better selection of redox indicators might have been provided; the quoted potentials of some are incorrect.

These are very minor shortcomings and in general this is an admirable and well thought out text which all teachers of analytical chemistry should find of great value.

R. BELCHER

Erotic tree



The Zieba tree, described in 1676 by Christopher Vielheuern. From *Bizarre Plants: Magical, Monstrous, Mythical* by William A. Emboden. Pp. ix+214. (Studio Vista, London, 1974.) £2.95.

See-through women replace yellow-bellied sapsuckers

Boston's Museum of Science. Open daily. Adults \$2, children \$1. Closed Thanksgiving, Christmas, New Year, July 4, Labour Day.

THE Museum of Science in Boston lives in one of those bleak, deserted areas peculiar to American cities. Although a mere five minutes' walk from the city centre the pedestrian finds himself hideously outnumbered by speeding cars. On a causeway across the Charles River, the buildings tempt you in by their very humanity amidst the heartlessness, and (again an American feature) an affluent and joyful interior in stark contrast to the external world. For the museum, whatever else it is, is full of joy, and the most un-museum like museum I have yet seen.

One colossal advantage it has over many other museums is its youth. When Bradford Washburn became director in 1939 it was sited in a four-square civil-war vintage building in downtown Boston, and as with so many museums of science, it was hard pressed for space and dominated by natural history exhibits. Washburn, a distinguished explorer and mountaineer, returned after war service to create a completely new multidisciplinary museum that would be particularly attractive to the young. In many ways he was lucky. The forties and early fifties were times when it was probably easier than today to persuade donors that science needed good publicity. Harvard Museum of Comparative Zoology, furthermore, served as a reference museum, releasing him from the burden of endless display cases of stuffed animals. The closeness of many educational, medical and technological institutions ensured ready assistance and advice (though is this true in London, say?). And there is Boston—just the right sort of city to plant this sort of thing in.

But if he was lucky, he has also had an incredible flair for what appeals to the young, and above all what makes them feel at home. As part of my preliminary research I took a couple of young boys—5 and 3—and let them loose. They found a lot that they enjoyed—a surprisingly large fraction of the exhibits—but eventually they gravitated to a huge tyre hanging, playground-style, from a ceiling. This gave them twenty minutes of simple fun; with them happily parked therein (indeed eventually almost impossible to prise out) I could look around at leisure. The next day, when I met Ned Pearce, the Museum's librarian, he had marked on a map for me what the most popular exhibits were—an

uncannily accurate description of Wilson's and Philip's somewhat erratic route through the museum. And the giant tyre? Yes that was on the list and Mr Pearce was mildly defensive about it, thinking I as a high priest of scientific orthodoxy would be cynical about a mere plaything. "You see we regard it as an important exhibit—children learn that there are things they can climb on here and not get screamed at. One of our key roles is to persuade kids that this is a friendly place and that people here are nice." This is put somewhat more sentimentally in one of their glossy brochures as "When [children] arrive . . . they are not only met by wonder and excitement and discovery. They are also met by love . . ." Mawkish stuff maybe but there is more than a grain of truth in it. A substantial fraction of the three quarters of a million visitors each year come in school buses, many of these from the inner city. They are naturally hostile to anything that smacks of authority and schoolroom formality, and for them a large number of volunteer guides—adults or teenagers—are laid on. It is essential, says Pearce, that guides, whether volunteer or full-time, should not be seen as policemen.

There has been much discussion in the museum on the social aspects of the educational programmes that they run, which include Saturday morning classes. Is the museum seen as a middle class institution perpetuating science as a middle class occupation? Questions like this (barely asked at all in England) are put in sharp focus when a busload of inner-city poor debouches into the arms of a potentially loving phalanx of suburban moms with their biology degrees and their worthy aims. As far as possible the museum tries to recruit volunteers and guides who can relate at first hand with the underprivileged but it is a difficult task. Already they are phasing out some of the Saturday activities which have tended to become high quality baby-sitting for the middle classes.

Once inside, how does the youngster experience the "enjoyment of learning" that Washburn dedicates the museum to? I think he does it in two ways—through the imaginative use of space, and through unashamed compromise. As museums go it has a very average amount of square footage per visitor. But there seemed to be less clutter and less pressure to circulate. Come on a rainy Sunday, said Pearce, then you'll see things differently, but I think the point is that if you go on a sunny

Wednesday you can really breathe.

And the compromise—it's not a poor man's university. You can mingle with a full sized *Tyrannosaurus rex* but the next exhibit along is a giant magnet one way, and a telephone exchange the other. The hot air balloon (for my money the best exhibit) rubs shoulders with the deep diving submarine. There is no logical order or historical succession to it all, it is a huge sampler. Washburn says "To touch a snake, to see liquid air or to pat a porcupine are never-to-be-forgotten experiences which will launch a youngster into a science career a thousand times more rapidly and powerfully than any number of carefully labelled exhibits".

In twenty years how has fashion changed in Boston? Natural history was and is unassailably Number One at all ages, declares Washburn. But whereas the prime interest used to be birds it is now 'me': the human being and how it works. Certainly the transparent woman, the stages in childbearing, the heart and the appendectomy draw more than such commonplaces of the seventies as lunar modules and computers, though the number who stay the course of ten realistic stages of removing an appendix without retiring slightly queasy is small.

The museum runs on an annual budget of around \$2 million of which about half is collected through admissions (including state-paid visits of school-children) and memberships. Private and corporate donors find the rest. Of course there is debate about the admission charge, although the staff fear for the crush on Sundays were entrance to be free. But at present that cannot even be considered.

Washburn has been successful at persuading the corporate donors to pay without visible returns. The museum is mercifully free of brand name exhibits which elsewhere range from the excellent, such as the IBM mathematics pavilion at Chicago, through the blatantly commercial "would you mind stepping into our exhibit and answering a five minute questionnaire on your cooked-ham eating habits?" also at Chicago to the dreary cutaway models of factories made by the apprentices and presented by the managing directors at—well, never mind where.

As long as Brad Washburn and his staff can continue to say 'no' if it isn't fun, I suspect that Boston will continue to be a hotbed of bright young scientists, many of whom will have experienced their first awakenings in or near the giant tyre.

DAVID DAVIES

obituary

Léon Rosenfeld

LÉON ROSENFELD was born at Charleroi, Belgium, on August 14, 1904. He studied physics and mathematics at the Université de Liège where he obtained a D.Sc. in 1926. He was associated with L. de Broglie and P. Langevin in Paris (1926–27) with M. Born in Göttingen (1927–29) and with W. Pauli in Zurich (1929–30). During this period he also collaborated with Th. De Donder in Brussels. In 1930 Rosenfeld visited Copenhagen, where he began a collaboration with Niels Bohr which lasted until Bohr's death in 1962. Rosenfeld joined the Université de Liège in 1930. From 1940 to 1947 he was Professor at the University of Utrecht and from 1947 to 1958 at the University of Manchester. In 1958 Rosenfeld became a professor at NORDITA.

Léon Rosenfeld was a member of the Royal Danish Academy, the Académie Royale de Belgique, the Académie Internationale d'Histoire des Sciences and the Académie Internationale de Philosophie des Sciences. He received the Prix Francqui, a high Belgian distinction, in Brussels in 1949.

The scientific interests of Léon Rosenfeld were unusually wide. They included quantum electrodynamics, nuclear physics, statistical mechanics, epistemology and history of sciences. His monographs *Nuclear Forces* (1948) and *Theory of Electrons* (1959) had a wide circulation.

His best known contribution is probably his fundamental paper with Niels Bohr in 1933 on the measurability of

electromagnetic quantities. In this paper Bohr and Rosenfeld showed that the only meaningful statements of quantum theory when applied to electrodynamics concern averages of field components over finite space time regions. In this way they proved the logical consistency of quantum electrodynamics.

Another series of well-known papers by Rosenfeld, mostly in collaboration with Humblet from Liège, concerns the theory of nuclear reactions (1962–65). These papers introduced a new and precise definition of resonant states.

All his life Rosenfeld was deeply interested in the foundations of physics. The 'Copenhagen' interpretation of quantum mechanics, still accepted by the majority of physicists, was developed by Bohr and Rosenfeld following the pioneering work of Heisenberg, Born and Bohr.

After Bohr's death Rosenfeld appeared as his natural spiritual heir. It would be a mistake, however, to consider Rosenfeld as simply the guardian of the Copenhagen orthodoxy. More than anyone else he knew that one of the main objects of theoretical physics is to define the precise domain of validity of concepts involved in the mathematical models of physical reality. He wrote, "No physical concept is sufficiently defined without the knowledge of its domain of validity". For this reason he devoted most of his efforts during the last years of his life to showing that the coherence of the rules of quantum mechanics can be tested explicitly through the study of the dynamics of large systems. For him the founda-

tions of quantum mechanics had not to be looked for in some axiomatic formulation valid for small systems with a discrete spectrum energy but, on the contrary, in the study of the limiting procedures involved in going to large systems. Since his early work he emphasised that there should exist a new type of complementarity between the "irreversible character of the macroscopic behaviour of the large systems and the time reversal invariance of its dynamical description at the atomic level". The outcome of these considerations is a generalised transformation theory which he developed in close collaboration with Prigogine's group in Brussels and in Austin. This transformation theory makes it possible to go from the usual representation of dynamics in terms of the Liouville-von Neumann operator L to a new representation in which the contributions responsible for irreversible processes are explicitly displayed.

His fatal illness brutally interrupted his contribution to the promising new development in theoretical physics.

For Rosenfeld science was a total engagement. Far from being a simple intellectual curiosity, Rosenfeld, like Bohr, "conceived science as a human endeavour to achieve harmony with nature, a common task to which it was the earnest duty of each individual scientist to contribute his share".

Rosenfeld will be remembered and missed by all his friends and scientific collaborators who had the privilege of knowing him and of appreciating his exceptional personal, moral and intellectual qualities.

Announcements

Appointments

M. A. Sleight has been appointed to the chair of biology at the **University of Southampton** (corrected announcement).

K. C. Calman has been appointed the Cancer Research Campaign Professor of Oncology at the **University of Glasgow**.

International meetings

September 10–11, **Energy Conservation Conference**, Glasgow (National Engineering Laboratory, East Kilbride, Glasgow G75 0QU).

September 23–26, **International Symposium on Contamination Control**, London (The Sam Black Organisation Ltd, 4 Harmsworth Way, London N20 8JU).

September 24, **Symposium on Human and Veterinary Trematode Infections**, London (Dr A. B. Simmonds, Chelsea College, Manresa Road, London SW3).

October 1–3, **1st National Topical Meeting on Nuclear Process Heat Applications**, New Mexico (Robert Y. Porton, Public Relations Office, Los Alamos Scientific Laboratory, PO Box 1663, Los Alamos, New Mexico 87544).

October 21, **Chemical and Mechanical Energy—Utilisation in Agriculture** (Assistant Secretary, Society of Chemical Industry, 14 Belgrave Square, London SW1X 8PS).

Erratum

In the article "Ionisation ledges in the equatorial ionosphere" by R. Raghavarao and M. R. Sivaraman (*Nature*, **249**, 331; 1974) the first sentence of the third paragraph should read, The cusps were observed on ISIS-2 ionograms from the dip latitude region 9°N to 7.5°S recorded at Ahmedabad on October 6, 1972.

The reference number for equation (1) should be 5 instead of 4.